# Harmine Derivatives, Intermediates Used in Their Preparation, Preparation Processes and Use thereof

Field of Invention

This invention belongs to the field of pharmaceutical compounds, and specifically relates to alkaloid compounds, and more specifically, to harmine derivatives of formula (I), intermediates used in their preparation, processes for preparing the same and uses thereof.

Description of the Prior Art

Harmine belongs to the family of β-carboline alkaloids, its chemical name is 7-methoxy-1-methyl-9H-pyrrole [3, 4-b] indole, and its molecular formula is $C_{13}H_{12}N_2O$. It has a molecular weight of 212.25 and a melting point of 261°C. The chemical structure of harmine is shown as follows :
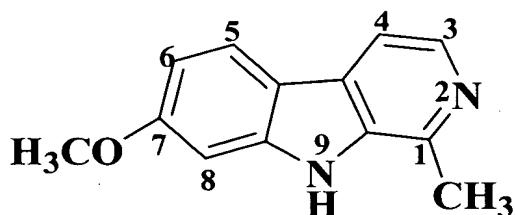
Harmine and its analogs thereof are widely distributed in nature. Numerous researches on the synthesis of harmine derivatives have been reported since harmine was first isolated from *Peganum harmala*. More than 300 harmine derivatives have been reported by far, and the number of new harmine derivatives still keeps increasing.

Previous reports and our preliminary investigation results demonstrated that harmine and its derivatives have significant antitumor activities, but also caused remarkbale acute neurotoxicity characterized by tremble, twitch, and jumping in experimental mice model. Results of investigation on the *in vitro* anti-tumor activity of harmine and its derivatives showed that these compounds had significant inhibition effect on several cultured tumor cell lines, such as Hela cells (cervical carcinoma), S-180 cells (sarcoma), BEL-7402 cells (hepatoma), MGC-803 cells (gastric carcinoma),

CNE2 cells (nasopharyngeal carcinoma), MA782'5S cells (breast cancer) and K562 cells (leucocythemia). Results of investigation on the *in vivo* antitumor activities of the total alkaloids and mixed alkaloids extracted from *Peganum harmala* plants, dominating ingredients of which were harmine and harmine derivatives, such as harmaline, harmalol and harman, displayed significant therapeutic effect on mice bearing Sarcoma (S180), reticulum cell sarcoma L2 and hepatoma. Moreover, these extracts exhibited significant synergistic effect when combined with cisplatin and adriamycin. However, neurotoxicities were the predominant acute toxic effects observed in mice receiving harmine and its derivatives, the acute toxic effects included tremble, twitch, erection of the tail and eclampsia. Death occurred mostly in the high dosage group. Survival animals would relieve and recover gradually in the next day after administration. Sub-acute toxicity test in rats showed that total alkaloids, extracted from *Peganum harmala* plants, can induce renal pathological change and kidney is the toxic target organ of the total alkaloids. Meanwhile, harmine and its derivatives do not exhibit obvious toxicity toward hemopoietic system, immune system, and reproductive system. What's more, long-term toxic side effects are not obvious.

Though present harmine and its analogs thereof have significant anti-tumor activity, they have also remarkable neurotoxicity. There are still no compounds having significant anti-tumor activity, together with low neurotoxicity, clinically used as anti-tumor medicaments.

Object of the Invention

The objects of this invention are to overcome the above defects of the prior art, and provide novel harmine derivatives with enhanced anti-tumor activity and lower neurotoxicity as well as easy preparation processes with high yields.

One of the objects of this invention is to provide novel harmine derivatives of formula (I).

Another object of this invention is to provide a process for preparing compounds of formula (I).

Another object of this invention is to provide a process for preparing 1,7,9-trisubstituted-β-carboline derivatives.

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Another object of this invention is to provide 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, esters and salts thereof.

Another object of this invention is to provide 9-substituted β-carboline-3-carboxylic acid, esters and salts thereof.

Another object of this invention is to provide a process for preparing ethyl 9-substituted 1-methyl-β-carboline-3-carboxylate

Another object of this invention is to provide a process for preparing 2,9-dibenzyl-β-carbolinium iodate.

Another object of this invention is to provide a process for preparing 2,9-dibenzyl-1-methyl-β-carbolinium bromate.

Another object of this invention is to provide intermediate compounds of formula (9a-16a) for the synthesis of said compounds.

Another object of this invention is to provide intermediate compounds of formula (9b-16b) for the synthesis of said compounds.

Another object of this invention is to provide intermediate compounds of formula (21a) for the synthesis of said compounds.

Another object of this invention is to provide intermediate compounds of formula (53a-55a) for the synthesis of said compounds.

Another object of this invention is to provide intermediate compounds of formula (10b) for the synthesis of said compounds.

Another object of this invention is to provide the use of said compounds in the manufacture of a medicament for treating tumors.

Another object of this invention is to provide the use of said compounds in the manufacture of a medicament, combined with phototherapy and radiation therapy, for treating tumors.

Summary of the Invention

Harmine derivatives of this invention have a structure of the following formula (I):
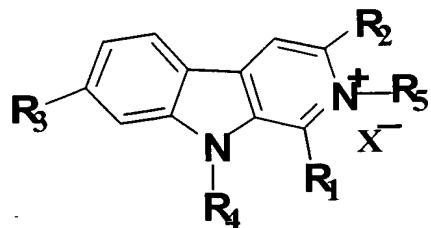
wherein

R_1 is hydrogen, C_{1-6} primary, secondary and tertiary linear or branched alkyl, C_{6-10} arylalkyl or 1-5 halogen, nitro or amino arylalkyl, heterocyclic group or alkenyl;

R_2 is hydrogen, carboxyl, ester group, carboxylate, acylamino, acyl halide group or C_{1-6} alkoxycarbonyl, aryloxycarbonyl, or heterocyclic oxycarbonyl;

R_3 is hydrogen, hydroxyl, C_{1-6} alkoxy, carboxylic esters, carboxylic salts, arylalkoxy, or heterocyclic oxy group;

R_4 is hydrogen, C_{1-6} alkyl, C_{1-6} hydroxyalkyl, C_{6-10} arylalkyl or 1-5 halogen arylalkyl, arylhydrocarbyl, arylcarboxyl, aryl ester group, arylamino group, arylnitro group, or heterocyclic group;

R_5 is hydrogen, C_{1-6} primary, secondary and tertiary linear or branched alkyl, C_{6-10} arylalkyl and 1-5 substituted arylalkyl, arylhydrocarbyl, arylcarboxyl, aryl ester group, arylamino group, arylnitro group, or heterocyclic group; and

R_1, R_2, R_3 and R_4 do not represent hydrogen at the same time, X^- is a halogen, sulfonic group, sulfuric group, or nitric acid group;

When R_2 and R_4 are hydrogen, R_1 is not methyl and R_3 is not methoxy;

When R_1 is methyl, R_2, R_3 and R_4 do not represent hydrogen at the same time;

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When R_1 is methyl, R_2 is hydrogen, and R_3 is methoxy, R_4 is not methyl, ethyl or butyl; and

When R_1 and R_3 are hydrogen, R_2 is not methoxycarbonyl and R_4 is not methyl.

In the compounds of the above formula (I), R_1 is preferably hydrogen or C_{1-4} alkyl or C_{6-8} arylalkyl.

In the compounds of the above formula (I), R_1 is preferably hydrogen or C_{1-2} alkyl.

In the compounds of the above formula (I), R_1 is most preferably hydrogen.

In the compounds of the above formula (I), R_2 is preferably hydrogen or C_{1-4} alkoxycarbonyl.

In the compounds of the above formula (I), R_2 is preferably hydrogen or C_{1-2} alkoxycarbonyl.

In the compounds of the above formula (I), R_2 is preferably ethoxycarbonyl.

In the compounds of the above formula (I), R_3 is preferably hydrogen, hydroxyl or C_{1-4} alkyloxy.

In the compounds of the above formula (I), R_3 is preferably hydrogen.

In the compounds of the above formula (I), R_4 is preferably hydrogen, C_{1-4} alkyl, C_{1-4} hydroxyalkyl or C_{6-8} arylalkyl or substituted arylalkyl.

In the compounds of the above formula (I), R_4 is preferably hydrogen, C_{1-2} alkyl, C_{1-2} hydroxyalkyl, or C_{6-8} arylalkyl or substituted arylalkyl.

In the compounds of the above formula (I), R_4 is preferably ethyl or benzyl.

In the compounds of the above formula (I), R_4 is preferably benzyl.

In the compounds of the above formula (I), preferably, R_1 is hydrogen, C_{1-4} alkyl or C_{6-8} arylalkyl, R_2 is hydrogen, hydroxyl, carboxyl, ester group, carboxylate, halogen or C_{1-4} alkoxycarbonyl, R_3 is hydrogen, hydroxyl, or C_{1-4} alkoxy, R_4 is hydrogen or C_{1-2} alkyl, C_{1-2} hydroxyalkyl, C_{6-8} arylalkyl or substituted arylalkyl, and R_5 is hydrogen, C_{1-6} primary, second, tertiary linear or branched alkyl and C_{6-10} arylalkyl and substituted arylalkyl.

In the compounds of the above formula (I), preferably, R_1 is hydrogen, R_2 is C_{1-2} alkoxycarbonyl, R_3 is hydrogen, and R_4 is C_{1-2} alkyl or C_{6-8} arylalkyl or substituted arylalkyl.

In the compounds of the above formula (I), preferably, R_1 is hydrogen, R_2 is ethoxycarbonyl, R_3 is hydrogen, and R_4 is ethyl or benzyl.

In the compounds of the above formula (I), preferably, R_1 is hydrogen, R_2 is ethoxycarbonyl, R_3 is hydrogen, and R_4 is benzyl.

In the compounds of the above formula (I), most preferably, R_1 is methyl, R_2 is ethoxycarbonyl, R_3 is hydrogen, R_4 is pentafluorobenzyl, and R_5 is hydrogen.

In the compounds of the above formula (I), most preferably, R_1 is hydrogen, R_2 is hydrogen, R_3 is hydrogen, R_4 is benzyl, R_5 is benzyl, and X is bromine.

A process for preparing the compound according to claim 1 of this invention comprises the following steps:

1) dissolving harmine (1) into an organic solvent or a mixed organic solvent;
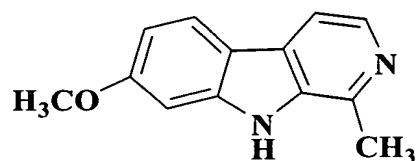
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2) adding 60% NaH and stirring it until there is no bubble formed;

3) adding halogenated alkane;

4) stirring and reacting said mixture at room temperature for 1 to 5

h; and

5) subjecting said mixture to conventional post-treatment and purification to produce 1,7,9-trisubstituted β-carboline derivatives.

A process for preparing the compound according to claim 1 of this invention comprises the following steps:

1) dissolving L-tryptophan and NaOH in water;

2) adding formaldehyde;

3) stirring and reacting said mixture at a temperature range from 0 °C to reflux for 1 to 6 h; and

4) subjecting said mixture to conventional post-treatment to produce 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (9a).

A process for preparing the compound according to claim 1 of this invention comprises the following steps:

1) dissolving β-carboline-3-carboxylate into an organic solvent or a mixed organic solvent;

2) adding 60% NaH and stirring it until there is no bubble formed;

3) adding halogenated alkane or halogenated aromatic alkane;

4) stirring and reacting said mixture at room temperature, or by heating for 2 to 5 h; and

5) subjecting said mixture to conventional post-treatment and purification to produce 9-substituted-β-carboline-3-carboxylates

A process for preparing the compound according to claim 1 of this invention comprises the following steps:

1) dissolving 1-substituted-β-carboline-3-carboxylate into an organic solvent;

2) adding 60% NaH and stirring it for 1 to 10 minutes;

3) adding halogenated alkane or halogenated aromatic alkane;

4) reacting said mixture at room temperature, or refluxing said mixture by heating; and

5) after the reaction is finished, subjecting said mixture to conventional post-treatment and purification to produce ethyl 9-substituted-1-methyl-β-carboline-3-carboxylates.

A process for preparing the compound according to claim 1 of this invention comprises the following steps:

1) mixing compound 10b of the following formula with glacial acetic acid,

10b
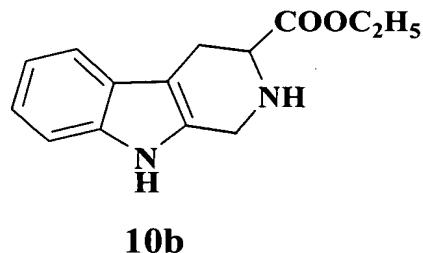
2) adding selenium dioxide;

3) refluxing said mixture by heating for 12 h; and

4) after the reaction is finished, subjecting the mixture to conventional post-treatment and purification to produce β-carboline.

A process for preparing the compound according to claim 1 of this invention comprises the following steps:

1) mixing compound 10 of the following formula with an organic solvent and 60% NaH;

10

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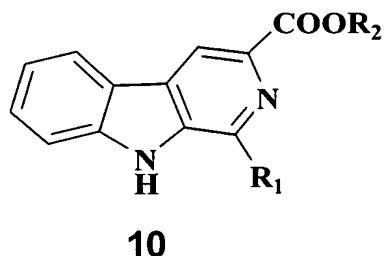
wherein R_1=H and R_2=C$_2$H$_5$;

2) stirring and reacting said mixture at room temperature for 1 to 10 minutes;

3) adding benzyl iodide;

4) stirring and reacting the mixture at a temperature of from 50 to 70°C for 1 to 5 h; and

5) subjecting the mixture to conventional post-treatment and purification to produce 2,9-dibenzyl-3-ethoxycarbonyl-β-carbolinium iodate.

A process for preparing the compound according to claim 1 of this invention comprises the following steps:

1) mixing compound 10 of the following formula with an organic solvent and 60% NaH;
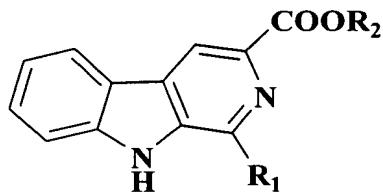

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wherein R_1=H and R_2=C$_2$H$_5$;

2) adding benzyl bromide;

3) stirring and reacting said mixture at a temperature of from 50 to 70°C for 1 to 10 h; and

5) subjecting the mixture to conventional post-treatment and purification to produce 2,9-dibenzyl-3-ethoxycarbonyl-β-carbolinium bromate.

A process for preparing the compound according to claim 1 of this invention comprises the following steps:

1) mixing compound 80 of the following formula with an organic solvent and 60% NaH;

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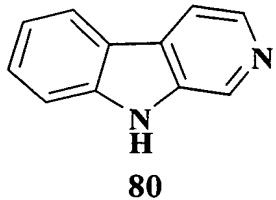
2) adding benzyl bromide or benzyl iodide;

3) stirring and reacting said mixture at a temperature of from 50 to 70℃ for 1 to 10 h; and

5) subjecting the mixture to conventional post-treatment and purification to produce 2,9-diphenylmethyl-β-carboline bromide or iodide salts.

This invention also prepares intermediates for the synthesis of the above compounds, i.e. compounds of the following formula (9a-16a):
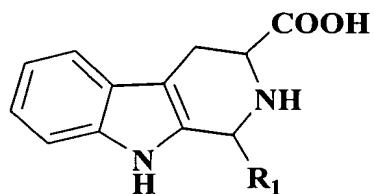
9a-16a

wherein

R_1 is methyl, ethyl, propyl, isopropyl, *n*-butyl, unsubstituted or halogenated phenyl, phenylmethyl, or phenylpropyl.

This invention also prepares intermediates for the synthesis of the above compounds, i.e. compounds of the following formula (9b-16b):
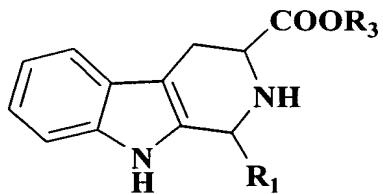
9b-16b

wherein
R_1 and R_3 are the same as R_1 in the compounds of formula (9a-16a)

as defined above.

This invention also prepares intermediates for the synthesis of the above compounds, i.e. compounds of the following formula (21a):
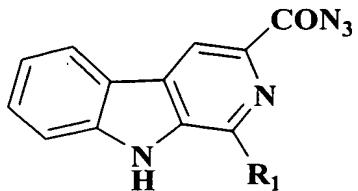
21a

wherein

R_1 is the same as R_1 in the compounds of formula (9a-16a) as defined above.

This invention also prepares intermediates for the synthesis of the above compounds, i.e. compounds of the following formula (53a-55a):
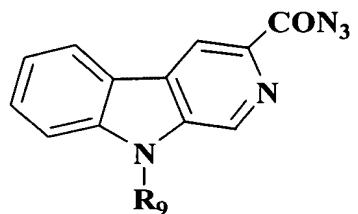
53a-55a

wherein

R_9 is methyl, ethyl, n-butyl, phenylmethyl, phenylpropyl, polyhalogenated phenylmethyl or polyhalogenated phenylpropyl.

This invention also prepares intermediates for the synthesis of the above compounds, i.e. compounds of the following formula (10b):
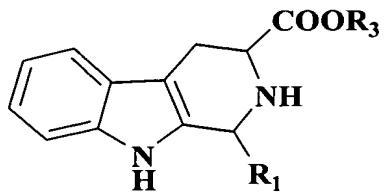

10b

wherein

R_1 and R_3 are the same as R_1 in the compounds of formula (9a-16a) as defined above.

Tests on the *in vitro* anti-tumor activity and studies on the *in vivo* anti-tumor therapeutic effect of the compounds of this invention showed that the compounds of this invention exhibit enhanced anti-tumor activities and lower neurotoxicities or no neurotoxicities. Moreover, processes for preparing the compounds of this invention are easy and have high yields. The compounds of this invention can be used for the manufacture of a medicament having low toxicity and high efficacy and useful for treating tumors.

It is demonstrated that the compounds of this invention, under UV excitation conditions, have photo induced DNA cleaving effect, which shows the structure-activity relationships between this kind of structure and anti-tumor activity. Therefore the compounds of this invention can also be used for the manufacture of a medicament for treating tumors in combination with phototherapy and radiation therapy.

Description of the figures

Figure 1 illustrates the photocleavage of supercoiled pGBK by β-carboline derivatives: wherein

Lane 1: DNA alone, lane 2: DNA+UV irradiation, lane 3: DNA+compound (1000 uM) without UV irradiation, lanes 4 to 10: DNA+compound+UV irradiation, the concentrations of the compound are respectively 1000, 300, 100, 30, 10, 3 and 1 (uM). Uppermost band: circular nicked DNA, middle band: linear DNA, and lowermost band: supercoiled DNA.

Figure 2 illustrates the effect of binding by β-carboline derivatives on the thermal stability of the CT-DNA.

Figure 3 illustrates the effect of absorbance by β-carboline derivatives on the UV spectrum of the CT-DNA.

Figure 4 illustrates the effect of β-carboline derivatives on the activity of DNA topoisomerase I in a cell free system.

The reaction system (20 ul) containing 35 mM Tris-HCl (pH 7.5), 50mM KCl, 5mM MgCl2, 1mM DTT, 2mM spermidine, 0.1mM EDTA, 50mg.1-1 BSA and 0.25ug supercoiled pGBK DNA and
1U topoisomerase I. Lane 1: DNA alone; lane 2: DNA+topoisomerase I, lane 3: DNA+topoisomerase I+250 uM

camptothecin, lanes 4 to 9 and 10 to 15, same as lane 2, but with 2000 uM, 600 uM, 200 uM, 60uM, 20uM and 6uM β-carboline derivatives, respectively. A represents compounds 80 and 81. B represents compounds 82 and 83. C represents compounds 37 and 36. D represents compounds 42 and 48. E represents compounds 49 and 66. Form I represents circular nicked DNA. Form II represents linear DNA. Form III represents supercoiled DNA.

Figure 5 illustrates the effect of β-carboline derivatives on the activity of DNA topoisomerase II in a cell free system.

The reaction system (20ul) containing 50mM Tris-HCl (pH8.0), 120mM KCl, 10mM $MgCl_2$, 1mM DTT, 0.5mM ATP, 30mg.l^{-1} BSA and 0.25ug supercoiled pGBK DNA and 1 U topoisomerase II. Lane 1: DNA alone, Lane 2: DNA+topoisomerase II, Lane 3 to 8 and 9 to 14, same as Lane 2, but with 2000uM, 600uM, 200uM, 60 uM, 20uM and 6uM β-carboline derivatives. A represents compounds 37 and 36. B represents compounds 49 and 66. C represents compounds 48 and 86. Form I represents circular nicked DNA. Form II represents linear DNA. Form III represents supercoiled DNA.

Figure 6 illustrates the FCM analysis of apoptosis of HepG2 cells induced by β-carboline derivative(Compound 60).

Figure 7 illustrates the TLC of harmine and 1,7,9-trisubstituted-β-carboline derivatives,

Wherein
Developing solvent: (Ethyl Acetate)
Detection wavelength: UV254 nm
Dots 1 to 8 represent compounds 1 to 8, respectively.

Figure 8 illustrates the FAB-MS spectrum of 9-phenylpropyl-7-methoxy-1-methyl-β-carboline.

Figure 9 illustrates the IR spectrum of 9-phenylpropyl-7-methoxy-1-methyl-β-carboline.

Figure 10 illustrates the UV spectrum of 9-phenylpropyl-7-methoxy-1-methyl-β-carboline.

Figure 11 illustrates the ^{1}H-NMR spectrum of 9-phenylpropyl-7-

methoxy-1-methyl-β-carboline.

Figure 12 illustrates the photomicrographs of β-carboline derivatives to human tumor cell HepG2

Figure 13 illustrates the anti-tumor effect of β-carboline derivatives on Lewis lung cancer.

Figure 14 illustrates the anti-tumor effect of β-carboline derivatives on S180 sarcoma.

Figure 15 illustrates the synthetic routes of the research of the modification to the structures of β-carboline derivatives.

Specific embodiments

Description of examples

Synthesis of 1,7,9-trisubstituted β-carboline derivatives

Experimental instruments and reagents

Experimental instruments:

RE-52C rotary evaporator (Hennan Yuhua Instrument Plant), SHZ-D (III) circulation vacuum water pump (Hennan Yuhua Instrument Plant), UV-8 UV array analysis meter (Wuxi Keda Instrument Plant), YRT-3 melting point apparatus (Precision Instrument Plant, Tianjin University), ZAB-HS double focusing magnetic mass spectrometer (VG Analytical, UK), Bruker Equinox 55 Fourier transform infrared spectrometer, KBr tablet, UV 2501PC UV spectrograph (Shimadzu, Japan), and Varian INOVA500 MRI spectrometer (Varian Inc. U.S.) were used. TMS is the internal standard, and $CDCl_3$ is the solvent.

Chemical reagents

Harmine 1 having a purity of 99%, provided by Xinjiang Huashidan Pharmaceutical Co. Ltd., methyl iodide (analytically pure, Zhejiang Yuhuan Biochemical Reagent Plant), ethyl iodide (analytically pure, Zhejiang Yuhuan Biochemical Reagent Plant), iodo-n-butane

(chemically pure, Shanghai Chemical Reagent Co., China National Pharmaceutical Group), 2-iodoethanol (Acros Organic, U.S.), benzyl bromide (chemically pure, Shanghai Chemical Reagent Co.), 1-bromo-phenylpropane (Acros Organic, U.S.), α-bromo-2,3,4,5,6-pentafluorobenzyl (Acros Organic, U.S.), and other domestically produced analytically pure or chemically pure reagents were used.

Synthetic route

2 R=CH$_3$

3 R=CH$_2$CH$_3$

4 R=n-C$_4$H$_9$

5 R=CH$_2$CH$_2$OH

6 R=CH$_2$ —⟨benzene⟩

7 R=CH$_2$ —⟨pentafluorobenzene⟩

8 R=(CH$_2$)$_3$ —⟨benzene⟩
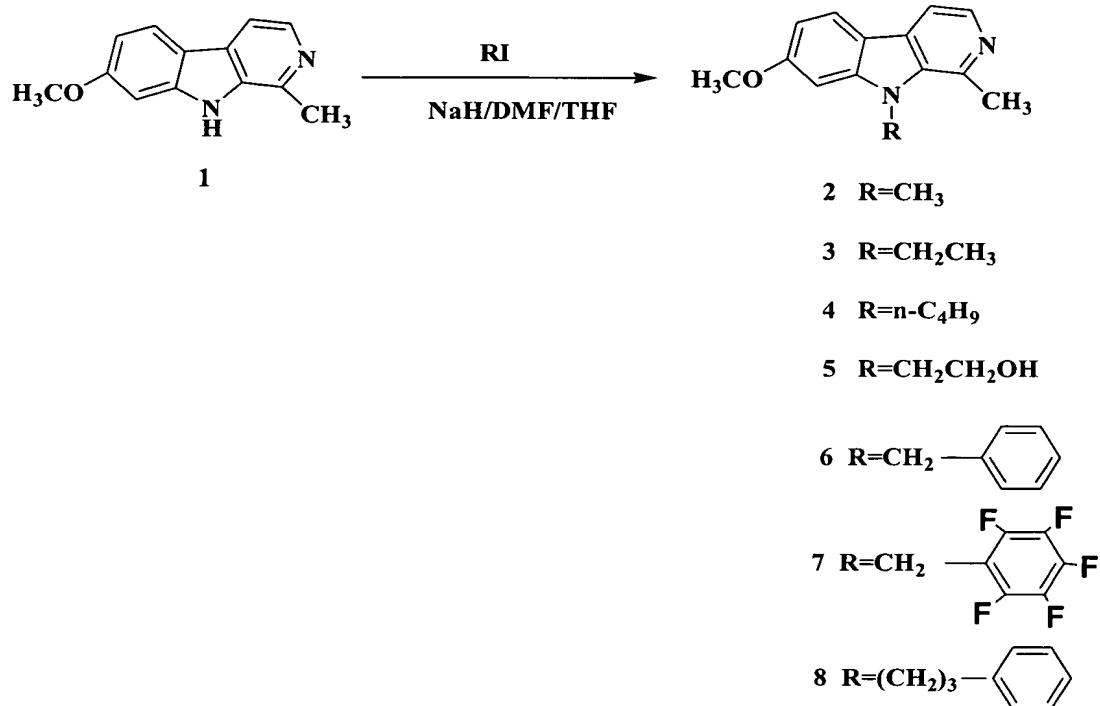
Operation steps

Example 1

General procedure for the preparation of 1,7,9-trisubstituted β-carboline derivatives

Harmine 1 (2.1g, 10mmol), DMF (50ml) and THF (50ml) were respectively added in a 250 ml round-bottom flask, and stirred at room temperature until the mixture became clear. Then 60% NaH (0.6g, 15mmol) was added and stirred until there were no bubbles formed. Alkyl halide (50mmol) was added dropwise. The mixture was stirred and reacted at room temperature for 5 h. THF was evaporated in reduced pressure, and the residues were poured into cold water. The mixture was adjusted to pH 3 with concentrated

hydrochloric acid and extracted with ethyl ether. The aqueous phase was neutralized with saturated $NaHCO_3$ solution and then extracted with ethyl acetate. The organic phase were combined, and washed with water and brine, then dried over anhydrous sodium sulfate, decolorized with activated carbon, filtered and evaporated in reduced pressure. The residue obtained was purified by silica gel column chromatography with ethyl acetate as the eluent. Upon recrystallization, crystals were obtained. Examples 2 to 5 were all treated according to the above procedures.

Example 2

Synthesis of 7-methoxyl-1,9-dimethyl-β-carboline (2): Afforded gray needle crystals (1.8g ,80%), mp 121-123℃.

Example 3

Synthesis of 7-methoxyl-9-ethyl-1-methyl-β-carboline (3): Afforded gray needle crystals (2.0g ,80%), mp 99-101℃.

Example 4

Synthesis of 7-methoxyl-9-n-butyl-1-methyl-β-carboline (4): Afforded gray needle crystals (2.1g ,78%), mp 104-105℃.

Example 5

Synthesis of 9-hydroxyethyl-7-methoxy-1-methyl-β-carboline (5): Afforded white crystals (0.7g ,54%), mp 204-206℃.

Example 6

Synthesis of 9-benzyl-7-methoxy-1-methyl-β-carboline (6)

Harmine 1 (2.1g, 10mmol), DMF (50ml) and THF(50ml) were respectively added in a 250ml round-bottom flask, and were stirred at room temperature for 10 minutes followed by the addition of 60% NaH (1.2g, 30mmol). The mixture was stirred until there were no bubbles formed. Benzyl bromide (3ml) was added dropwise. The mixture was then refluxed by heating for 8 h. THF was evaporated in reduced pressure, and the residues were poured into cold water. The mixture was adjusted to pH 3.0 with concentrated HCl, and extracted with ethyl ether. The aqueous phase was neutralized (pH8)

with saturated NaHCO₃ solution and extracted with ethyl acetate. The organic phases were combined, washed with water and brine, dried over anhydrous sodium sulfate, filtered, evaporated in reduced pressure and then recrystallized with ethyl acetate to afford gray crystals (2.2g, 67%). mp 131-133℃.

Example 7

Synthesis of 9-(2',3',4',5',6'-pentafluoro)benzyl-7-methoxy-1-methyl -β-carboline (7)

Harmine 1 (0.53g, 2.5mmol), 15 ml of DMF and 15ml of THF were respectively added in a 100 ml round-bottom flask, and were stirred at room temperature until the mixture became clear. 60% NaH (0.3g, 7.5mmol) was added and stirred until there were no bubbles formed. α-Bromo-2,3,4,5,6-pentafluorobenzyl (0.5ml) was added dropwise. The mixture was stirred and reacted at room temperature for 1 hour. Later the mixture was treated in a manner to that described for compound **2** to afford gray needle crystals (0.64g , 65%), mp 173-174℃.

Example 8

Synthesis of 9-phenylpropyl-7-methoxy-1-methyl-β-carboline (8):

Harmine 1 (0.53g, 2.5mmol), DMF (15ml) and THF (15ml) were respectively added in a 100 ml round-bottom flask, and stirred at room temperature until the mixture became clear, then 60% NaH (0.3g, 7.5mol) was added and stirred until there were no bubbles formed. 1-Brombenzenepropropane (2ml) was added dropwise. The mixture was refluxed for 12 h. THF was evaporated in reduced pressure. The residues were poured into 30ml cold water and extracted with ethyl acetate. The organic phases were combined, and washed with water and brine, dried over anhydrous sodium sulfate, filtered, evaporated in reduced pressure, and the residue dissolved in 50 anhydrous ethanol. The pH was adjusted to 4 with concentrated HCl, the mixture were concentrated in vacuum and then recrystallized with acetone to afford yellow solids. The solids were dissolved into a mixed solution of water and ethyl acetate. The pH was adjusted to 8 with saturated NaHCO₃ solution. The organic layer was isolated, and the aqueous phase was extracted with ethyl acetate. The organic phases were combined, washed with water and brine, dried over anhydrous sodium sulfate, decolorized

with activated carbon, filtered, concentrated, and recrystallized with ethyl ether to afford gray needle crystals (0.42g, 51%), mp 117-118℃.

Physico-chemical constants, TLC and spectra analyses of 1,7,9-substituted β-carboline derivatives

Table 6   Physico-chemical data of 1,7,9-substituted β-carboline derivatives

Compd	Formula	FW	Yield (%)	Appearance	Solubility	Mp (℃)
1	$C_{13}H_{12}N_2O$	212	---	gray needle crystal	Soluble in organic solvents, such as alcohol, ether, and ester, and water-insoluble	260-261
2	$C_{14}H_{14}N_2O$	226	80	gray needle crystal	Soluble in organic solvents, such as alcohol, ether, and ester, and water-insoluble	121-123
3	$C_{15}H_{16}N_2O$	240	83	gray needle crystal	Soluble in organic solvents, such as alcohol, ether, and ester, and water-insoluble	99-101
4	$C_{17}H_{20}N_2O$	268	78	gray needle crystal	Soluble in organic solvents, such as alcohol, ether, and ester, and water-insoluble	104-105
5	$C_{15}H_{16}N_2O_2$	256	62	white crystal	Soluble in organic solvents, such as alcohol, ether, and ester, and water-insoluble	204-206
6	$C_{20}H_{18}N_2O$	302	54	gray needle crystal	Soluble in organic solvents, such as alcohol, ether, and ester, and water-insoluble	131-133
7	$C_{15}H_{11}F_5N_2O$	392	65	gray needle crystal	Soluble in organic solvents, such as alcohol, ether, and ester, and water-insoluble	173-174
8	$C_{20}H_{22}N_2O$	330	51	gray needle	Soluble in organic solvents, such as	117-118

18

				crystal	alcohol, ether, and ester, and water-insoluble	

Table 7 FAB-MS, IR and UV data of 1,7,9-substituted β-carboline derivatives

Compd	Formula	FAB-MS m/e (M+1)	IR (KBr, cm^{-1})	UV ($\lambda_{max,}$ nm)
1	$C_{13}H_{12}N_2O$	213	ND	ND
2	$C_{14}H_{14}N_2O$	227	3451,3380,2744,1628,1570, 1469,1348,1249,1152,1045, 810	345,332,302, 264,244,213
3	$C_{15}H_{16}N_2O$	241	3362,3128,1622,1564,1497, 1451,1346,1262,1217,1136, 1095,812	344,332,302, 264,243,213
4	$C_{17}H_{20}N_2O$	269	3428,2959,2927,2863,1884, 1621,1563,1497,1448,1356, 1242,1197,1137,812	346,333,302, 265,244,213
5	$C_{15}H_{16}N_2O_2$	257	3294,2696,1629,1569,1467, 1352,1155,1051,810	327,304,248, 210
6	$C_{20}H_{18}N_2O$	303	3421,2958,1620,1565,1498, 1447,1404,1361,1256,1197, 1172,1044,825	396,342,330, 301,244,209
7	$C_{15}H_{11}F_5N_2O$	393	2961,2836,1622,1502,1446, 1256,1174,1122,1026,974 816	337,325,300, 240,208
8	$C_{20}H_{22}N_2O$	330	3052,2995,2931,2666,1881, 1623,1563,1449,1408,1238, 1156,1043,801,744,702	345,332,302, 265,244,210

Table 8 ^{1}H-NMR data of 1,7,9-substituted β-carboline derivatives

Compd	^{1}H-NMR (δ, CDCl$_3$)

2	8.24-8.25(1H,m,H-5),7.93-7.96(1H,m,H-3),7.69-7.71(1H,m,H-4),6.86-6.89(1H,m,H-8),6.81-6.83(1H,m,H-6),4.04-4.07(3H, m, NCH_3,),3.94-3.95(3H,m,OCH_3), 3.04-3.05(3H,m,Ar-CH_3)
3	8.26-8.27(1H,d,J=5Hz,H-5),7.96-7.98(1H,d,J=5Hz,H-3),7.74-7.75(1H,d, J=4.5Hz,H-4),6.86-6.90(2H,m,H-8,H-6),4.52-4.57(2H,m,CH_2CH_3),3.95(3H,s, OCH_3),3.05(3H,s,CH_3),1.43-1.46(3H,m,CH_2CH_3)
4	8.26-8.28(1H,d,J=5.5Hz,H-5),7.95-7.97(1H,d,J=9Hz,H-3),7.71-7.72(1H,d,J= 5Hz,H-4),6.85-6.88(2H,m,H-8,H-6),4.43-4.46(2H,m,J=8Hz,$CH_2CH_2CH_2CH_3$), 3.94(3H,s,OCH_3),3.01(3H,s, Ar-CH3),1.78-1.84(2H,m,$CH_2CH_2CH_2CH_3$),1.41-1.48 (2H,m,$CH_2CH_2CH_2CH_3$),0.97-1.00 (3H,m,$CH_2CH_2CH_2CH_3$)
5	8.15-8.16(1H,d,J=4Hz,H-5),7.93-7.94(1H,d,J=3.5Hz,H-3),7.63-7.64(1H,d,J= 5Hz,H-4) ,6.88-6.95 （2H,m,H-8,H-6） ,4.66-4.71 （2H,m,NCH_2CH_2OH） ,4.06-4.08 （2H,m,NCH_2CH_2OH） ,3.94(3H,s,OCH_3),2.99(3H,s,CH_3)
6	8.29-8.30(1H,d, J=5.5Hz,H-5),8.01-8.02(1H,d,J=8.5Hz,H-3),7.80-7.81(1H,d, J= 5.5Hz,H-4), 7.23-7.30(3H,m,H-8,H-6,Ar-H),6.98-7.00(1H,d,J=7.0Hz,Ar-H), 6.90-6.92(1H,m, Ar-H),6.76(1H,s,Ar-H),5.75(2H,s,NCH_2Ar),3.85(3H,s,OCH_3), 2.88(3H,s, CH_3)
7	8.32-8.33(1H,d,J=5Hz,H-5),7.94-7.95(1H,d,J=7.5Hz,H-3),7.72-7.73(1H,d,J= 7.5Hz,H-4),7.26(1H,s,H-8),6.87-6.89(1H,m,H-6),5.85(2H,s,CH_2Ar), 3.88(3H,s, OCH_3), 3.05(3H,s,Ar-CH_3)
8	8.25-8.26(1H,d,J=5Hz,H-5),7.92-7.94(1H,d,J=8.5Hz,H-3),7.69-7.70(1H,d,J= 5.0Hz,H-4),7.29-7.32(2H,m,H-8,H-6),7.20- 7.25(3H,m,Ar-H), 6.84-6.86(1H,m, Ar-H),6.63-6.64(1H,m,Ar-H),4.42-4.45(2H,m,$NCH_2CH_2CH_2Ar$),3.84-3.85(3H, ,OCH_3),2.88(3H,s,CH_3),2.74-2.77(2H,m,$NCH_2CH_2CH_2Ar$),2.12-2.18(2H,m, $NCH_2CH_2CH_2Ar$)

Spectra analyses of typical compounds

See figures 8 to 11 for the spectrum analyses of compound 8.

Synthesis of 3- and 1,3-disubstituted β-carboline alkaloids

Experimental instruments and reagents

Experimental instruments are as defined above.

Chemical reagents

L-tryptophan (Acros Organic, U.S.), formaldehyde solution (analytically pure, Guangzhou Chemical Reagent Plant), 40% acetaldehyde (analytically pure, Shanghai Chemical Reagent Co., China National Pharmaceutical Group), propionaldehyde (chemically pure, Shanghai Chemical Reagent Co., China National Pharmaceutical Group), n-butyraldehyde (analytically pure, Shanghai Chemical Reagent Co., China National Pharmaceutical Group), benzaldehyde (analytically pure, Guangzhou Chemical Reagent Plant), 4-methoxy benzaldehyde (analytically pure, Shanghai Chemical Reagent Co., China National Pharmaceutical Group) and p-hydroxy benzaldehyde (analytically pure, Shanghai Shuangxi Flavor Adjuvant Plant), and domestically manufactured analytically pure or chemically pure reagents were used.

Synthetic routes and operational steps

Scheme I

9a R_1=H
11a R_1=CH$_3$
12a R_1=CH$_2$CH$_3$
13a R_1=CH$_2$CH$_2$CH$_3$

14a R_1=

15a R_1= —OCH$_3$

16a R_1= —OH

9b R_1=H R_2=CH$_3$
10b R_1=H R_2=CH$_2$CH$_3$
11b R_1=CH$_3$ R_2=CH$_2$CH$_3$
12b R_1=CH$_2$CH$_3$ R_2=CH$_2$CH$_3$
13b R_1=CH$_2$CH$_2$CH$_3$ R_2=CH$_2$CH$_3$

14b R_1= R_2=CH$_3$

15b R_1= —OCH$_3$ R_2=CH$_3$

16b R_1= —OH R_2=CH$_3$

9 R_1=H R_2=CH$_3$
10 R_1=H R_2=CH$_2$CH$_3$
11 R_1=CH$_3$ R_2=CH$_2$CH$_3$
12 R_1=CH$_2$CH$_3$ R_2=CH$_2$CH$_3$
13 R_1=CH$_2$CH$_2$CH$_3$ R_2=CH$_2$CH$_3$

14 R_1= R_2=CH$_3$

15 R_1= —OCH$_3$ R_2=CH$_3$

16 R_1= —OH R_2=CH$_3$
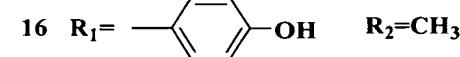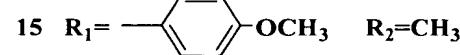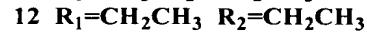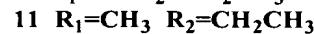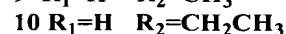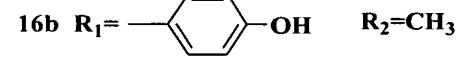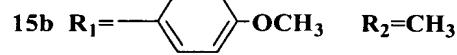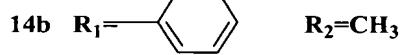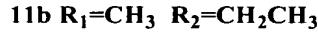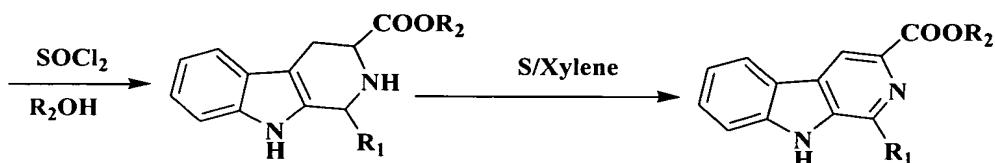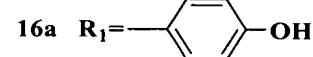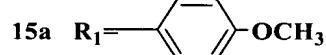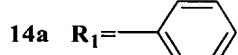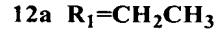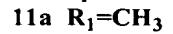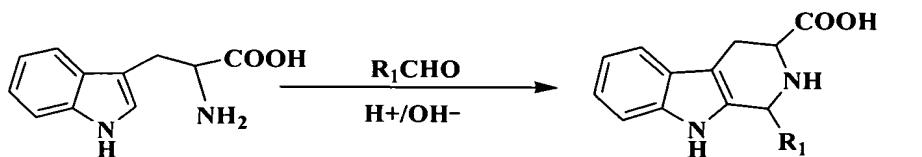
Example 9

Synthesis of 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (9a)

L- tryptophan (10.2g, 50mmol), NaOH (2.0g, 50mmol) and H$_2$O (20ml) were added in a 250 ml round-bottom flask, and were stirred until the mixture became clear followed by the addition of formaldehyde (37%, 50mmol). The mixture was stirred and reacted at room temperature for 3 h. After being refluxed for another 3 h, the mixture was poured into 200ml cold water. While being stirred, the mixture was adjusted to pH 6 with 5N HCl. White solids were precipitated and then stored at 4°C overnight. The white solids

were filtered, washed well with water and a small amount of methanol, dried, and recrystallized with methanol to obtain white solids (9.2g, 85%), mp 308-309℃ (reference: 309-310℃).

Example 10

General procedure for the preparation of 1,2,3,4-tetrahydro-β-carboline-3-carboxylates

1,2,3,4-Tetrahydro-β-carboline-3-carboxylic acid 9a (50mmol), methanol or ethanol (250ml) and thionyl chloride (10ml) were added into a 500 ml round-bottom flask. The mixture was refluxed for 1 to 2 h. After alcohol was evaporated in reduced pressure, 100ml cold water was added. The pH was adjusted to 9 with saturated NaHCO₃ solution. Then the mixture was extracted with ethyl acetate. The organic phases were combined, washed with water and brine, dried over anhydrous sodium sulfate, filtered, concentrated in vacuum. Recrystallization was conducted with ethyl acetate. Examples 11 and 12 were treated according to the above procedures.

Example 11

Synthesis of methyl 1,2,3,4-tetrahydro-β-carboline-3-carboxylate (9b): white solids were obtained, and the yield was 57%.

Example 12

Synthesis of ethyl 1,2,3,4-tetrahydro-β-carboline-3-carboxylate (10b): white needle crystals were obtained, and the yield was 63%.

Example 13

General procedure for the preparation of β-carboline-3-carboxylates

Compound 9b-10b (50mmol), anhydrous xylene (200ml) and sulfur (200mmol) were added into a 100 ml round-bottom flask. The mixture was refluxed for 12 h and then cooled to room temperature. The mixture was stored at 4℃ overnight to precipitate light yellow crystals. After filtration and wash with a small amount of cold xylene and wash liberally with petroleum ether, the solids were dissolved into 2000 ml of a corresponding alcohol. The solids were decolorized with activated carbon and filtered with 200 to 300

meshes silica gel. The filtrate was concentrated in vacuum and recrystallized with a corresponding alcohol. Examples 14 and 15 were treated according to the above procedures.

Example 14
Synthesis of methyl β-carboline-3-carboxylate (9): white solids were obtained, the yield was 66%, mp 308-309℃.

Example 15
Synthesis of ethyl β-carboline-3-carboxylate (10): white solids were obtained, the yield was 77%, mp 230-231℃ (reference [1]: 231-232℃).

Example 16

Synthesis of 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (11a)

L- tryptophan (5.10g, 25mmol), H_2SO_4 (0.01M, 30ml) and 40% acetaldehyde (9ml) were added in a 250 ml round-bottom flask, and then stirred and reacted at room temperature for 8 h. After filtration, wash with water and drying, white solids (4.0g, 69%) were obtained.

Example 17

Synthesis of 1-ethyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (12a)

L- tryptophan (5.10g, 25mmol), water (300ml), H_2SO_4 (0.05M, 30 ml) and propionaldehyde (8ml) were added in a 250 ml round-bottom flask, and then stirred and reacted at room temperature for 24 h. After filtration, wash with water and drying, white solids (4.5g, 74%) were obtained.

Example 18

Synthesis of 1-propyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (13a)

L- tryptophan (5.10g, 25mmol), water (300ml), H_2SO_4 (0.5M, 50 ml), n-butyraldehyde (10ml) and ethanol (100ml) were added in a 250 ml round-bottom flask, and then stirred and reacted at room

temperature for 24 h. After filtration, wash with water and drying, white solids (3.75g, 58%)were obtained.

Example 19

General procedure for the preparation of ethyl 1-alkyl-1,2,3,4-tetra-hydro-β-carboline-3-carboxylates

1-Alkyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid 11a-13a (20 mmol), and anhydrous ethanol (250ml) were added in a 250 ml round-bottom flask. Redistilled thionyl chloride (6ml) was carefully added. The mixture was refluxed for 1 h. Ethanol was then evaporated in reduced pressure to afford white solids. The white solids were dissolved in 100ml water. Saturated $NaHCO_3$ was used to conduct neutralization. Extraction was conducted with ethyl acetate. Organic phases were combined, washed with water and brine, dried with anhydrous sodium sulfate, decolorized with activated carbon, filtered, concentrated in vacuum and recrystallized with ethyl acetate. Examples 20 to 22 were treated according to the above procedures.

Example 20

Synthesis of ethyl 1-methyl-1,2,3,4-tetrahydro-β-carboline -3-carboxylate (11b): white solids were obtained, and the yield was 71%.

Example 21

Synthesis of ethyl 1-ethyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate (12b): white solids was obtained, and the yield was 52%.

Example 22

Synthesis of ethyl 1-propyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate (13b): white solids were obtained, and the yield was 84%.

Example 23

General procedure for the preparation of ethyl 1-alkyl-β-carboline-3-carboxylates

Ethyl 1-alkyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate 11b-13b (20mmol), sulfur (60mmol) and xylene (200ml) were added into a 500ml round-bottom flask and then refluxed for 10 h. The mixture was then cooled to room temperature. After being stored at 4℃ overnight, light yellow solids were precipitated. After filtration and wash with a small amount of cold xylene and wash well with petroleum ether, the solids were dissolved into 2000 ml of anhydrous ethanol. The solids were decolorized with activated carbon and filtered with 200 to 300 meshes silica gel. The filtrate was concentrated in vacuum and recrystallized with ethyl acetate. Examples 24 to 26 were conducted according to the above operation steps.

Example 24

Synthesis of ethyl 1-methyl-β-carboline-3-carboxylate (11): white solids were obtained, the yield was 48%, and mp 217-218℃.

Example 25

Synthesis of ethyl 1-ethyl-β-carboline-3-carboxylate (12): white solids were obtained, the yield was 47%, and mp 209-210℃.

Example 26

Synthesis of ethyl 1-propyl-β-carboline-3-carboxylate (13): white solids were obtained, the yield was 58%, and mp 194-195℃.

Example 27

General procedure for the preparation of 1-aryl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acids

L- tryptophan (50mmol), a corresponding aromatic aldehyde (55 mmol), H_2SO_4 (0.5M, 50ml), H_2O (150ml) and ethanol (100ml) were added in a 550ml round-bottom flask, and then refluxed for 5 h. After adding concentrated ammonia (100ml), the mixture was refluxed for another hour. Ethanol was evaporated. The resulting solution was cooled and extracted with ethyl ether. The aqueous phase was concentrated to 50 ml and was adjusted to pH 5, and the precipitate were then filtered, washed well with water and dried. Examples 28 to 30 were conducted according to the above

operational steps.

Example 28

Synthesis of 1-phenyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (14a): white solids were obtained, and the yield was 98%.

Example 29

Synthesis of 1-(4-methoxyphenyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (15a): white solids were obtained, and the yield was 82%.

Example 30

Synthesis of 1-(4-hydroxyphenyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (16a): light yellow solids were obtained, and the yield was 94%.

Example 31

General procedure for the preparation of 1-aryl-1,2,3,4-tetrahydro-β-carboline-3-carboxylates

1-Aryl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid 14a-16a (50 mmol) and methanol (250 ml) were respectively added into a 500 ml round-bottom flask. Redistilled thionyl chloride (20 ml) was carefully added. The mixture was refluxed for 2 h. Methanol was evaporated in reduce pressure. After filtration and wash with a small amount of acetone, ash gray solids were obtained. The solids were dissolved in 300 ml cold water and adjusted to pH 9. The mixture was extracted with ethyl acetate. After wash with water and brine, the organic phase was dried over anhydrous sodium sulfate and then recrystallized with ethyl acetate. Examples 32 to 34 were treated according to the above procedures.

Example 32
Methyl 1-phenyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate (14b): white solids were obtained, and the yield was 95%.

Example 33
Methyl 1-(4-methoxyphenyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylate (15b): white solids were obtained, and the yield was 90%.

Example 34
Methyl 1-(4-hydroxyphenyl)-1,2,3,4-tetrahydro-β-carboline-3-
carboxylate (16b): white solids were obtained, and the yield was 85%.

Example 35
General procedure for the preparation of of methyl
1-aryl-β-carboline-3-carboxylates

Methyl 1-aryl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate 14b-16b
(20 mmol), sulfur (60 mmol), and xylene (200 ml) were respectively
added into a 500 ml round-bottom flask. The mixture was refluxed for
18 h and then cooled to room temperature. After being stored at 4℃
overnight, light yellow solids were precipitated. After filtration and
wash with a small amount of cold xylene and wash well with
petroleum ether, the solids were dissolved into 1000 ml of ethyl
acetate, decolorized with activated carbon and filtered with 200 to
300 meshes silica gel. The filtrate was concentrated in vacuum and
recrystallized with ethyl acetate. Examples 36 to 38 were conducted
according to the above operation steps.

Example 36

Synthesis of methyl 1-phenyl-β-carboline-3-carboxylate (14): white
solids were obtained, the yield was 69%, and mp 257-258℃.

Example 37

Synthesis of methyl 1-(4-methoxyphenyl)-β-carboline-3-carboxylate
(15): white solids were obtained, the yield was 63%, and mp 229-230
℃.

Example 38

Synthesis of methyl 1-(4-hydroxyphenyl)-β-carboline-3-carboxylate
(16): white solids were obtained, the yield was 56%, and mp 267-269
℃.

Scheme II

a) NaOH, EtOH; HCl b) SOCl$_2$,n-BuOH; c) CHCl$_3$,MeOH
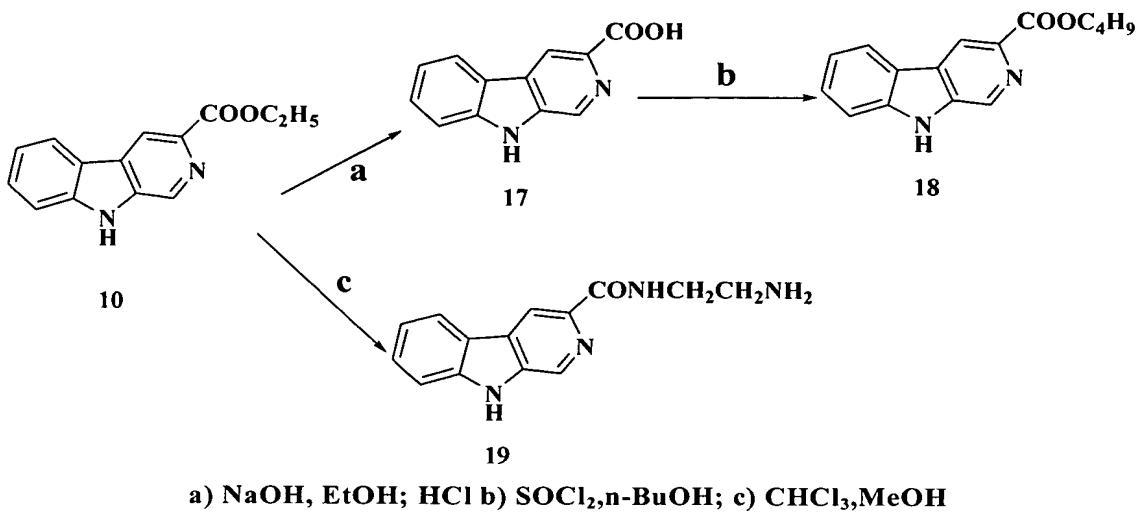
Example 39

Synthesis of β-carboline-3-carboxylic acid (17)

Compound 10 (1.2g, 5mmol), NaOH (0.8g, 20mmol), ethanol (20ml) and H$_2$O (40ml) were added into a 50 ml round-bottom flask. The mixture was refluxed for 2 h. Ethanol was then evaporated in reduced pressure. The mixture was adjusted to pH with 5M HCl. After cooling with cold water, filtration, wash well with water and recrystallization with ethanol, white solids (0.96g, 90%) were obtained. and mp 307-309°C (reference [1]: 310°C).

Example 40

Synthesis of butyl β-carboline-3-carboxylate (18)

Compound 17 (2.1g, 10mmol), NaOH (0.8g, 20mmol), n-butanol (100 ml) and thionyl chloride (5ml) were added into a 250 ml round-bottom flask. The mixture was refluxed for 6 h. Excessive n-butanol was then removed in reduced pressure. The residues were dissolved in water followed by the addition of ethyl acetate. While being stirred, the mixture was adjusted to pH 8 with NaHCO$_3$ solution. The organic layer was isolated. The aqueous phase was extracted with ethyl acetate. The organic phases were combined, washed with water and brine, dried over anhydrous sodium sulfate, decolorized with activated carbon and concentrated in vacuum. The residues were dissolved in ethyl acetate, and purified by silica gel column chromatography with ethyl acetate as the eluent, the recrystallized

with ethyl ether/petroleum ether (2:5) to afford white needle crystals(1.8g, 67%), and mp 211 -212℃(reference [1]: 210-211℃).

Example 41

Synthesis of 3-ethylamino-β-carboline-3-formamide (19)

Ethylenediamine (24ml, 27mmol) was added into a dry 250 ml three-neck round-bottom flask and heated to 80-90℃. While the mixture was stirred, compound 10 (2.4g, 10mmol) was added dropwise and dissolved in a solution of 40ml chloroform and 30ml methanol for about 1 h. The mixture was refluxed for 10 h, and the solvent was evaporated. A mixed solution of 50ml chloroform and 20ml water was added into the residues. After being stored at 4℃ overnight, light yellow solids were precipitated. After filtration and drying, white needle crystals (0.85g, 30%) were obtained. and mp 233-236℃ (reference [1], 234-237℃, 25%).

Synthetic route III

22 R$_1$=CH$_3$

23 R$_1$=CH$_2$CH$_3$
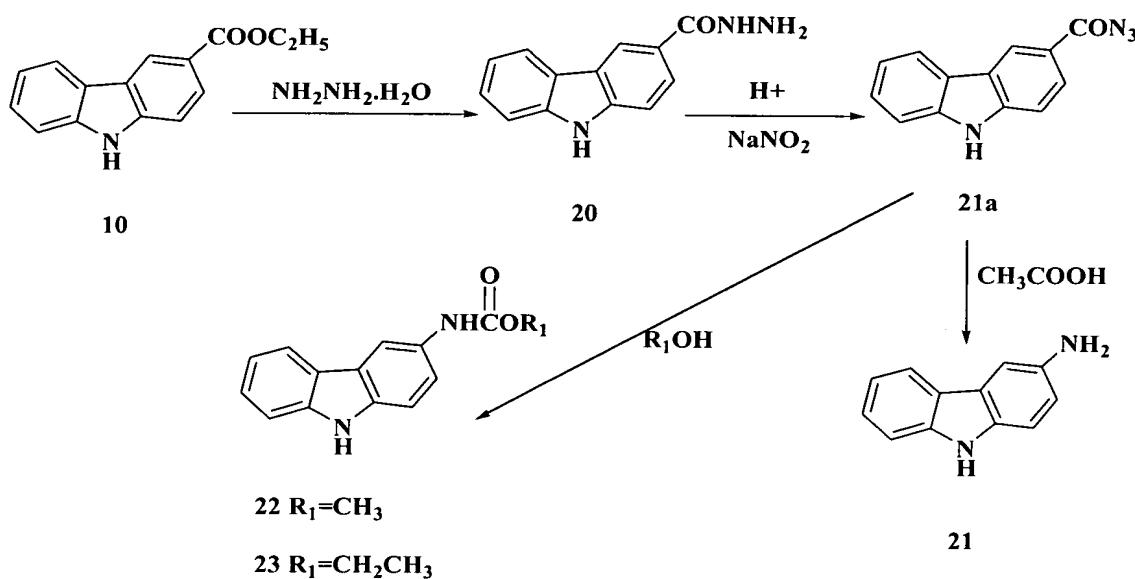
Example 42

Synthesis of β-carboline-3-carbohydrazine (20)

Ethyl β-carboline-3-carboxylate (10) (2.4 g, 10 mmol) was dissolved in ethanol (50 ml) followed by adding 85% hydrazine hydrate (15 ml). The mixture was refluxed for 6 h and concentrated to 30 ml in

30

reduced pressure. After cooling, filtration, wash with ethanol, and natural drying in the air, white solids were obtained (2.0g, 80%), Samples for analysis could be recrystallized with 90% ethanol to form white flaring crystals, and mp 289-290℃ (reference [100]: 289-291℃).

Example 43

Synthesis of 3-(azidocarbonyl)-β-carboline (21a)

Concentrated HCl (1.0ml) was added dropwise into a mixed suspension formed from compound 20 (2.0g, 2.9mmol) and water (50 ml). The light yellow solution was cooled in an ice bath to 0℃, and then an aqueous solution (10ml) of nitrous acid (0.2g, 2.9mmol) was added dropwise to react with the light yellow solution at 0℃ for 30 minutes. The mixed reaction solution was then alkalified with a saturated $NaHCO_3$ solution. Solids were collected by filtration, washed by water and vacuumly dried to afford light yellow solids (0.51g, 77%). Said solids were apt to be decomposed, and further purification was not necessary and used directly for the next steps.

Example 44

Synthesis of 3-amino-β-carboline (21)

Compound 21a (0.6g, 2.5mmol) was dissolved in a solution of 30 ml water/glacial acetic acid (1: 1). The mixture was refluxed for 1h. Accompanied with the formation of carbon dioxide gas, the raw materials was gradually disappeared. Then the solvent was evaporated in reduced pressure. The solid residues were recrystallized by ethyl acetate and then filtered to obtain yellow sheet-like crystals (0.35g, 77%), and mp 288-290℃ (reference [100]: 289 to 291℃).

Example 45

Synthesis of 3-[(methoxycarbonyl)amino]-β-carboline (22)

Compound 21a (0.2g, 0.84mmol) was dissolved in methanol (50ml). The mixture was refluxed for 10 h. The reaction mixtrue was cooled, concentrated to 40 ml in vacuum, recrystallized and filtered to obtain white solids (0.12g, 60%). Samples for analysis could be recrystallized with ethanol, and mp 180-182℃, and then decomposed at 230℃.

Example 46

Synthesis of 3-[(ethoxycarbonyl)amino]-β-carboline (23)

Compound 21a (0.2g, 0.84mmol) was dissolved in ethanol (50ml). The mixture was refluxed for 10 h. The reaction liquid was cooled and concentrated to 40 ml in vacuum. After recrystallization and filtration, white solids (0.12g, 60%) were obtained. Samples for analysis could be recrystallized with ethanol, and mp 222-224°C.

Synthetic route IV

a) LiBH₄, THF; b) HAc
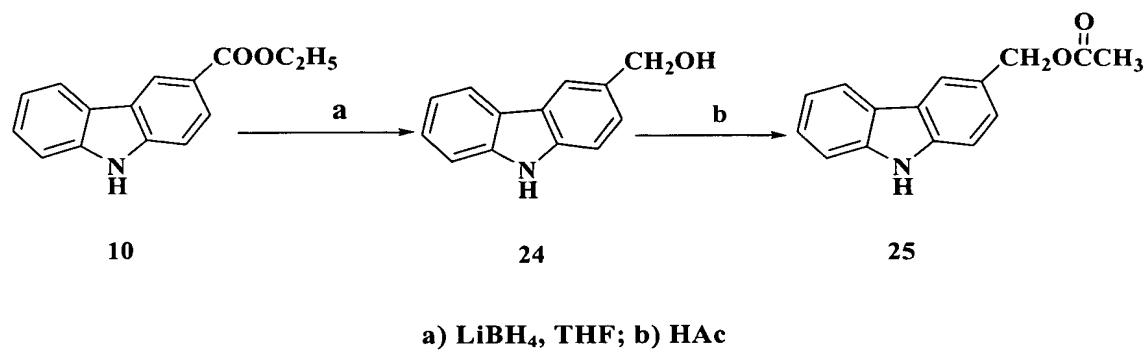
Example 47

Synthesis of 3-hydroxymethyl-β-carboline (24)

Compound 10 (7.0g, 31mmol) was dissolved in anhydrous THF (900 ml) followed by the addition of LiBH₄ (3.4g, 155mmol). The mixture was stirred at room temperature for 9 h and then cooled. Water (100ml) was added into the mixture and stirred overnight. Then the solvent was removed in reduced pressure. With the addition of water (500ml), extraction was conducted with dichloromethane (1 L) and then with ethyl acetate. The organic phases were combined, concentrated in vacuum and purified by silica gel column chromatography with ethyl acetate/methane (3: 1) as the eluent to afford white solids (5.0 g, 82%), and mp 228-230°C (reference [2]: 225-228°C).

Example 48

Synthesis of 3-acetylmethoxy-β-carboline (25)

Compound 24 (1.98g, 10mmol) was mixed with acetic acid (50ml).

The mixture was refluxed for 2 h. The solvent was removed in reduced pressure. Water (100ml) was added. The mixture was extracted with ethyl acetate. The organic phases were combined, washed with water and brine, dried over anhydrous sodium sulfate, concentrated in vacuum, and recrystallized with ethyl ether to afford white needle crystals (2.2g, 92%), and mp 131-132℃.

Physico-chemical properties, TLC and spectra analyses of 3- and 1,3-substituted-β-carboline derivatives

Table 9 Physico-chemical data of 3- and 1,3-substituted-β-carboline derivatives

Compd	Formula	FW	Yield (%)	Appearance	Solubility	Mp (℃)
9	$C_{13}H_{10}N_2O_2$	226	66	white solids	soluble in alcohols, esters etc.	259-260
10	$C_{14}H_{12}N_2O_2$	240	77	white solids	soluble in alcohols, esters etc.	230-231
11	$C_{15}H_{14}N_2O_2$	254	48	white solids	soluble in alcohols, esters etc.	217−218
12	$C_{16}H_{16}N_2O_2$	268	47	white solids	soluble in alcohols, esters etc.	209−210
13	$C_{17}H_{18}N_2O_2$	282	58	white solids	soluble in alcohols, esters etc.	194−195
14	$C_{19}H_{14}N_2O_2$	302	69	white solids	soluble in alcohols, esters etc.	257−258
15	$C_{20}H_{16}N_2O_3$	332	63	white solids	soluble in alcohols, esters etc.	229−230
16	$C_{19}H_{14}N_2O_3$	318	56	white solids	soluble in alcohols, esters etc.	267−269
17	$C_{12}H_8N_2O_2$	212	90	light yellow solids	soluble in alcohols and DMSO	307−309
18	$C_{16}H_{16}N_2O_2$	268	67	white solids	soluble in alcohols, esters etc.	211−212
19	$C_{14}H_{14}N_4O$	254	30	white solids	soluble in alcohols and	233−236

					DMSO	
20	$C_{12}H_{10}N_4O$	226	80	white flaring crystals	slightly soluble in alcohols, soluble in DMSO	289—290
21	$C_{11}H_9N_3$	183	77	yellow solids	slightly soluble in alcohols, soluble in DMSO	289—291
22	$C_{13}H_{11}N_3O_2$	241	60	white solids	soluble in alcohols, esters etc.	180-182
23	$C_{14}H_{13}N_3O_2$	255	60	white solids	soluble in alcohols, esters etc.	221-223
24	$C_{12}H_{10}N_2O$	198	82	white solids	soluble in alcohols, esters etc.	226-228
25	$C_{14}H_{12}N_2O_2$	240	92	white needle-like crystals	soluble in alcohols, ethers, esters, chlorofom etc.	131-132

Table 10 FAB-MS, IR and UV data of 3- and 1,3-substituted-β-carboline derivatives

Comp	Formula	FAB-MS m/e(M+1)	IR (KBr, cm^{-1})	UV$_{\lambda max}$ (nm)
9	$C_{13}H_{10}N_2O_2$	227	3258, 1724, 1627, 1502, 1434 1341, 1301, 1251, 1100, 1022	ND
10	$C_{14}H_{12}N_2O_2$	241	ND	ND
11	$C_{15}H_{14}N_2O_2$	255	3316, 3041, 2978, 1709, 1567, 1499, 1367, 1344, 1254, 1145, 1031	345,330,303, 270,236,219, 204
12	$C_{16}H_{16}N_2O_2$	269	3327, 2974, 2930, 1705, 1566, 1498, 1451, 1346, 1257, 1143, 1043	345,331,303, 270,236
13	$C_{17}H_{18}N_2O_2$	283	3329, 2963, 1706, 1567, 1498, 1367, 1344, 1252, 746	346,331,302, 271,237
14	$C_{19}H_{14}N_2O_2$	303	3315, 1720, 1623, 1350, 1251, 1215, 1098, 739	355,344,279, 231
15	$C_{20}H_{16}N_2O_3$	333	3639, 3320, 1714, 1611, 1512, 1351, 1255, 1103, 1033, 833,	357,347,284, 268,230,215
16	$C_{19}H_{14}N_2O_3$	319	3459, 3159, 1715, 1691, 1610, 1513, 1432, 1352, 1260, 839,	387,340,327, 285,215
17	$C_{12}H_8N_2O_2$	213	2250-3750,1630,1585,1372, 1219,752	356,337,288, 282,234,212
18	$C_{16}H_{16}N_2O_2$	269	3233,2957,2868,1708,1627, 1553,1501,1462,1339,1304, 1248,1102	ND

19	$C_{14}H_{14}N_4O$	255	ND	ND
20	$C_{12}H_{10}N_4O$	227	ND	ND
21	$C_{11}H_9N3$	184	ND	ND
22	$C_{13}H_{11}N_3O_2$	242	ND	ND
23	$C_{14}H_{13}N_3O_2$	256	ND	ND
24	$C_{12}H_{10}N_2O$	199	ND	ND
25	$C_{14}H_{12}N_2O_2$	241	3371,2938,1743,1692,1616, 1450,1372,1238,1053	326,315,284, 259,230,207

Note: ND represents that said assessment was not conducted.

Table 11 ^{1}H-NMR data of 3- and 1,3-substituted-β-carboline

derivatives

Compd	1H-NMR（δ，CDCl3）
11	10.50(1H,s,NH),8.78(1H,s,H-4),8.15-8.17(1H,d,J=8Hz,H-8),7.58-7.60(1H,d,J=8Hz, H-5), 7.52- 7.55 (1H,m,H-6),7.30-7.33(1H,m,H-7), 4.44-4.48(2H,m,OCH$_2$CH$_3$),2.68 (3H,s,CH$_3$), 1.32-1.35 (3H,m, OCH$_2$CH$_3$)
12	9.63(1H,s,NH),8.76(1H,s,H-4),8.16-8.17(1H,d,J=8Hz,H-8),7.60-7.62(1H,d,J=8.5Hz,H-5),7.5 4-7.57(1H,m,H-6),7.31-7.34(1H,m,H-7),4.47-4.51(2H,m,OCH$_2$CH$_3$),3.11-3.15(2H,m,CH$_2$C H$_3$),1.40-1.43(3H,m,OCH$_2$CH$_3$),1.29-1.32(3H,m,CH$_2$CH$_3$)
13	11.14(1H,s,-NH),8.79(1H,s,H-4),8.15-8.17(1H,d,J=7.5Hz,H-8),7.64-7.66(1H,d,J=8Hz,H-5), 7.51-7.54(1H,m,H-6),7.25-7.31(1H,m,H-7),4.42-4.46(2H,m,OCH$_2$CH$_3$),2.76-2.79 (2H, m, ArCH$_2$CH$_2$CH$_3$), 1.45-1.51 (2H,m,ArCH$_2$CH$_2$CH$_3$), 1.25-1.32 (3H, m, OCH$_2$CH$_3$), 0.37-0.43 (3H,m,CH$_2$CH$_2$CH$_3$)
14	8.91(1H,s,NH),8.86(1H,s,H-4),8.20-8.21(1H,d,J=8Hz,H-8),7.90-7.91(2H,m,H-5,H-6) 7.58-7.60 (2H,m,H-7,Ar-H), 7.54-7.57(2H,m,Ar-H), 7.41-7.44 (1H, m, Ar-H), 7.35-7.37 (1H,m,Ar-H), 4.04(3H,s,OCH$_3$)
15	9.40(1H,s,NH),8.76(1H,s,H-4),8.15-8.16(1H,d,J=8Hz,H-8),7.69-7.70(2H,d,J=8.5Hz,H-5, H-6),7.54-7.56(2H,m,H-7,Ar-H),7.31-7.34(1H,m,Ar-H),6.75-6.77(2H,d,J=8.5Hz,Ar-H),4.00(3H,s,OCH$_3$),3.69(3H,s,Ar-OCH$_3$)
16	9.51(1H,s,NH),8.83(1H,d,J=8Hz,H-4),8.20-8.21(1H,d,J=8Hz,H-8),7.83-7.85(2H,d,J=8.5Hz, H-5,H-6),7.60-7.61(2H,m,H-7,Ar-H),7.38-7.39(1H,m,Ar-H),7.03-7.04(2H,d,J=8Hz,Ar-H), 4.06(3H,s,OCH$_3$)
18	11.74(1H,s,NH),9.29(1H,s,H-4),8.89(1H,s,H-1),8.20-8.22(1H,d,J=8.0Hz,H-8),7.86-7.87(1H, d,J=8.5Hz,H-5),7.58-7.62(1H,m,H-6),7.33-7.36(1H,m,H-7),4.51-4.54(2H,m,CH$_2$CH$_2$CH$_2$- CH$_3$),1.81-1.87(2H,m,CH$_2$CH$_2$CH$_2$CH$_3$),1.48-1.56(2H,m,CH$_2$CH$_2$CH$_2$CH$_3$),0.97-1.00(3H,m,

	CH₂CH₂CH₂CH₃)
25	9.47 (1H, s, NH), 9.03 (1H, s, H-4), 8.32-8.34 (1H, d, J=8Hz, H-1),8.26-8.28 (1H, d, J=8.5Hz, H-8), 8.19-8.20 (2H, m, H-5), 7.67-7.70 (1H, m, H-6), 7.47-7.51 (1H, m, H-7), 5.29 (2H, s, CH₂), 2.16 (3H, s, CH₃)

Synthesis of 3,9-disubstituted-β-carboline derivatives

Experimental instruments and materials

Experimental instruments are as described above.

Chemical reagents

NaH (Merck-Schuchardt Co. Germany), methyl iodide (analytically pure, Zhenjiang Yuhuan Biological Reagent Plant), ethyl iodide (analytically pure, Zhenjiang Yuhuan Biological Reagent Plant), iodo-*n*-butane (chemically pure, Shanghai Chemical Reagent Co., China National Pharmaceutical Group), 1-bromine-3-phenyl propane (Acros Organic, U.S.), α-bromine-2,3,4,5,6-pentafluorobenzyl (Acros Organic, U.S.), 2-bromine-acetyl benzophenone (Acros Organic, U.S.), 2-bromine-4-phenyl-acetyl benzophenone (Acros Organic, U.S.), tetrahydro potassium aluminium (Acros Organic, U.S.), and other domestically manufactured analytically pure or chemically pure reagents were used.

Synthetic routes and operational steps

Scheme I

9

DMF/ THF

NaH, RI or RBr

26 R=CH₃
27 R=CH₂CH₃
28 R=n-C₄H₉
29 R=CH₂C₆H₅
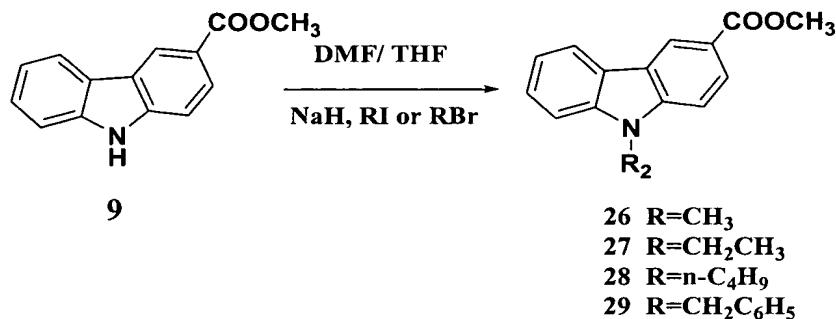
Example 49

General procedure for the preparation of 9-substituted-β-carboline-3-carboxylate

β-carboline-3-carboxylate (10mmol), DMF (50ml) and THF (50ml) were respectively added in a 250 ml round-bottom flask, and were stirred until the mixture became clear at room temperature. NaH (50mmol) was added and stirred until there were no bubbles formed. Alkyl halide or aromatic halide (60mmol) was added dropwise. The mixture was stirred to react at rom temperature or by heating for 5 h. After the reaction was finished, THF was removed in reduce pressure, and 200ml 2N HCl solution was added. The mixture was extracted with toluene. The aqueous phase was neutralized with saturated NaHCO₃ and extracted with ethyl acetate. The organic phases were combined, washed with water and brine, dried, filtered and concentrated in vacuum. The residues were prurified by silica gel column chromatography with ethyl acetate as the eluent, and recrystallized with ethyl ether/petroleum ether. Examples 50 to 61 were treated according to the above procedures.

Example 50

Synthesis of methyl 9-methyl-β-carboline-3-carboxylate (26): Afforded white needle crystals (1.8g, 75%), and mp 215-216℃.

Example 51

Synthesis methyl 9-ethyl-β-carboline-3-carboxylate (27): Afforded white needle crystals (2.0g, 79%), and mp 155-156℃.

Example 52

Synthesis of methyl 9-n-butyl-β-carboline-3-carboxylate (28): Afforded white needle crystals (2.3g, 82%), and mp 181-183℃.

Example 53

Synthesis of methyl 9-benzyl-β-carboline-3-carboxylate (29): Afforded white needle crystals (2.3g, 73%), and mp 187-188℃.

Scheme II

Reagents: DMF/ THF, NaH, RI or RBr

10	30 R=CH$_3$	34 R=CH$_3$
	31 R=CH$_2$CH$_3$	35 R=CH$_2$CH$_3$
	32 R=n-C$_4$H$_9$	36 R=n-C$_4$H$_9$
	33 R=CH$_2$C$_6$H$_5$	37 R=CH$_2$C$_6$H$_5$
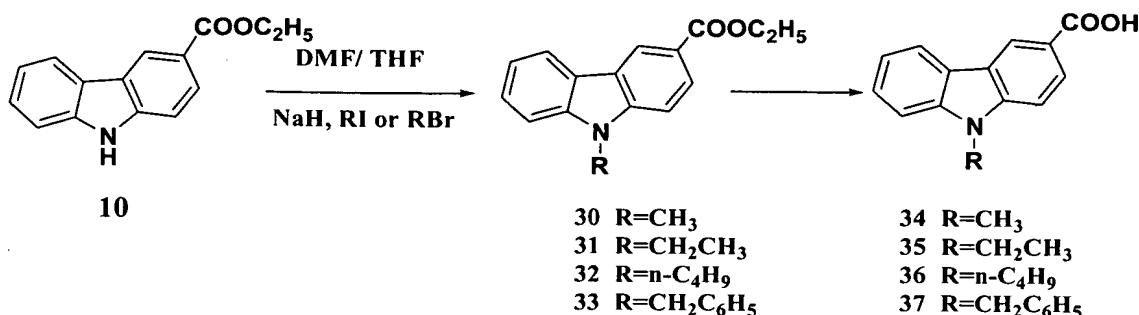
Example 54

Synthesis of ethyl 9-methyl-β-carboline-3-carboxylate (30): white needle crystals (1.9g, 75%) were obtained, mp 139-140℃.

Example 55

Synthesis of ethyl 9-ethyl-β-carboline-3-carboxylate (31): white needle crystals (1.8g, 67%) were obtained, mp 117-118℃.

Example 56

Synthesis of ethyl 9-butyl-β-carboline-3-carboxylate (32): white needle crystals (2.3g, 78%), mp 76-77℃.

Example 57

Synthesis of ethyl 9-benzyl-β-carboline-3-carboxylate (33):

Ethyl β-carboline-3-carboxylate 10 (4.8g, 20mmol), DMF (100ml) and THF (100ml) were respectively added in a 100ml round-bottom flask, and were stirred at room temperature for 15 minutes, then 60% NaH (2.4g, 60mmol) was added and stirred until there were no bubbles formed. Benzyl bromide (15ml) was added, and the mixture was refluxed for 12 h. Later the mixture was treated in a manner similar to that described for compound 29 to afford white crystals (4.6g, 70%), mp 126-127℃.

Scheme III
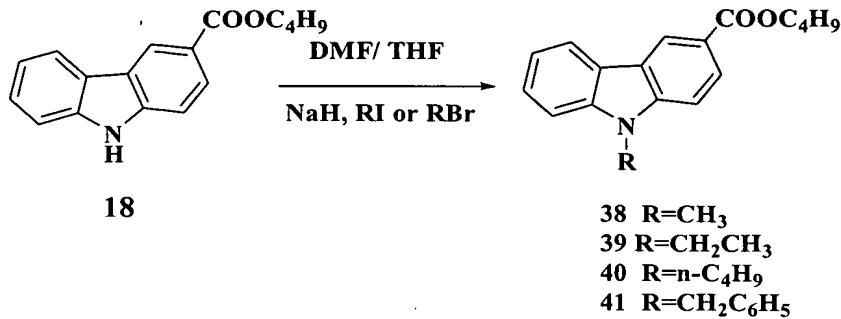
Example 58

Synthesis of butyl 9-methyl-β-carboline-3-carboxylate (38): white needle crystals (2.0g, 70%) were obtained, mp 235- 238°C.

Example 59
Synthesis of butyl 9-ethyl-β-carboline-3-carboxylate (39): white needle crystals (2.0g, 65%) were obtained, mp 86-88°C.

Example 60
Synthesis of butyl 9-butyl-β-carboline-3-carboxylate (40): 2.2 g white needle crystals were obtained, the yield was 74%, and mp 94-95°C.

Example 61

Synthesis of butyl 9-benzyl-β-carboline-3-carboxylate (41): white crystals (1.0g, 67%) were obtained, mp 104-105°C.

Example 62

General procedure for the preparation of 9-substituted-β- carboline -3-carboxylic acid

9-Substituted-β-carboline-3-carboxylate (20mmol), 200 ml of water, 100 ml of ethanol and NaOH (4.0g, 100mmol) were added in a 250 ml round-bottom flask. The mixture was refluxed for 2 h and then cooled. The pH was adjusted to 6 with 5N HCl. After removing ethanol in reduced pressure, light yellow solids were precipitated which were then cooled, filtered, washed with water and dried. Examples 63 to 66 were conducted according to the above operational steps.

Example 63

Synthesis of 9-methyl-β-carboline-3-carboxylic acid (34): light yellow solids were obtained, the yield was 99%, and mp 267-269℃.

Example 64

Synthesis of 9-ethyl-β-carboline-3-carboxylic acid (35): light yellow solids were obtained, the yield was 98%, and mp 201-202℃.

Example 65

Synthesis of 9-butyl-β-carboline-3-carboxylic acid (36)

Compound 32 (3.0g, 10mmol), 100ml of water, 50ml of ethanol, and NaOH (2.0g, 50mmol) were added into a 100 ml round-bottom flask. The mixture was refluxed for 2 h. The subsequent operational steps were conducted according to those for synthesizing compound 3 to obtain light yellow solids (2.7g, 99%), and mp 182-184℃.

Example 66

Synthesis of 9-benzyl-β-carboline-3-carboxylic acid (37): yellow solids were obtained, the yield was 94%, and mp 261-262℃.

Scheme IV
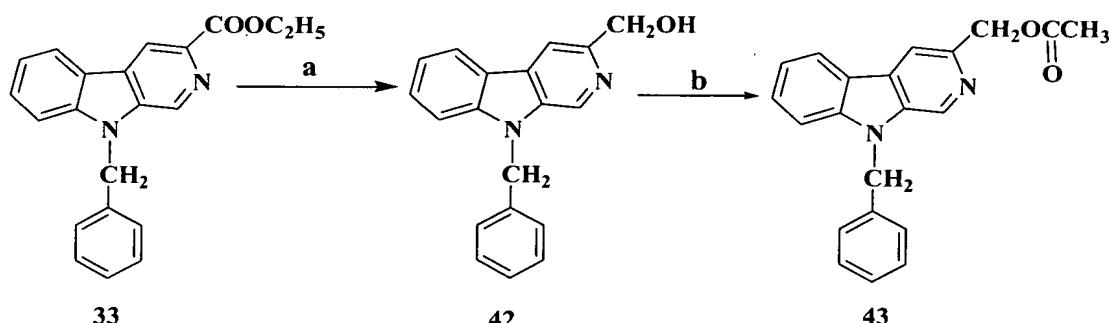
a) LiAlH4, THF; b) HAc

Example 67

Synthesis of 9-benzyl-3-hydroxymethyl-β-carboline (42)
Compound 33 (3.3g, 10mmol) was mixed with anhydrous THF (100 ml). The mixture was added dropwise to a mixed solution of LiAlH$_4$ (1.2g, 30mmol) and anhydrous THF (100ml). After that, the mixture was refluxed for 10 h. Then the mixture was cooled to room

temperature. 10% NaOH (50ml) was added into the mixture and then stirred for 20 minutes. The mixture was extracted with ethyl acetate, the organic phases were combined and washed with water and brine, dried over anhydrous sodium sulfate, filtered, evaporated and purified by silica gel column chromatography with ethyl acetate as the eluent. Upon recrystallization, white solids (1.5g, 52%) were obtained, mp 120-122℃.

Example 68

Synthesis of 9-benzyl-3-acetylmethoxy-β-carboline (43)

Compound 42 (1.44g, 5mmol) was mixed with acetic acid (50ml). The mixture was refluxed for 2 h. After the reaction was finished, the solvent was evaporated in reduced pressure. Water (100ml) was added into the residues, and then the mixture was extracted with ethyl acetate. The organic phases were combined, washed with water and brine, dried over anhydrous sodium sulfate, concentrated in vacuum, and recrystallized with ethyl acetate, white solids (1.5g,94%) were obtained, mp 141-142℃.

Scheme V
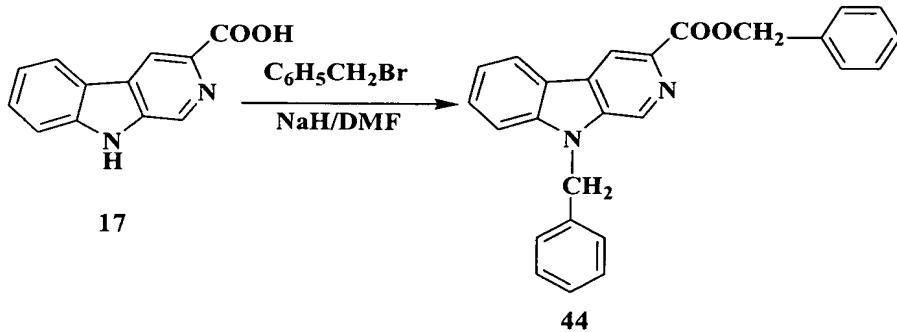
Example 69

Synthesis of benzyl 9-benzyl-β-carboline-3-carboxylate (44)

Compound 17 (2.12g, 10mmol) was mixed with DMF (50ml). After stirring the mixture at room temperature for 30 minutes, NaH (1.6g, 40mmol) was added and stirred until the solution became clear. After that, benzyl bromide (5ml) was added. The mixture was stirred and reacted at room temperature for 1 h. The reaction mixture was poured into cold water (200 ml) and the mixture was extracted with ethyl acetate. The organic phases were combined and washed with water

and brine, then the organic phase was evaporated in reduced pressure, the residue was dissolved in anhydrous ethanol, and then the mixture was adjusted to pH 4 with concentrated HCl, and evaporated and recrystallized with acetone/ethyl ether, light yellow solids were obtained. The light yellow solids were dissolved in a mixed solution of water and ethyl acetate. The pH was adjusted to 8 with saturated $NaHCO_3$ solution. The organic phases were isolated. The aqueous layer was extracted with ethyl acetate. After drying, decolorization with activated carbon, filtration, concentration, and recrystallization with ethyl ether, white solids (2.3g, 57%) were obtained, mp 169-170 °C.

Scheme VI

45 R=(CH₂)₃—⟨phenyl⟩

46 R=CH₂—⟨pentafluorophenyl⟩

47 R=CH₂—C(=O)—⟨phenyl⟩

48 R=(CH₂)₃ —⟨phenyl⟩

49 R=CH₂—⟨pentafluorophenyl⟩

50 R=CH₂—C(=O)—⟨phenyl⟩
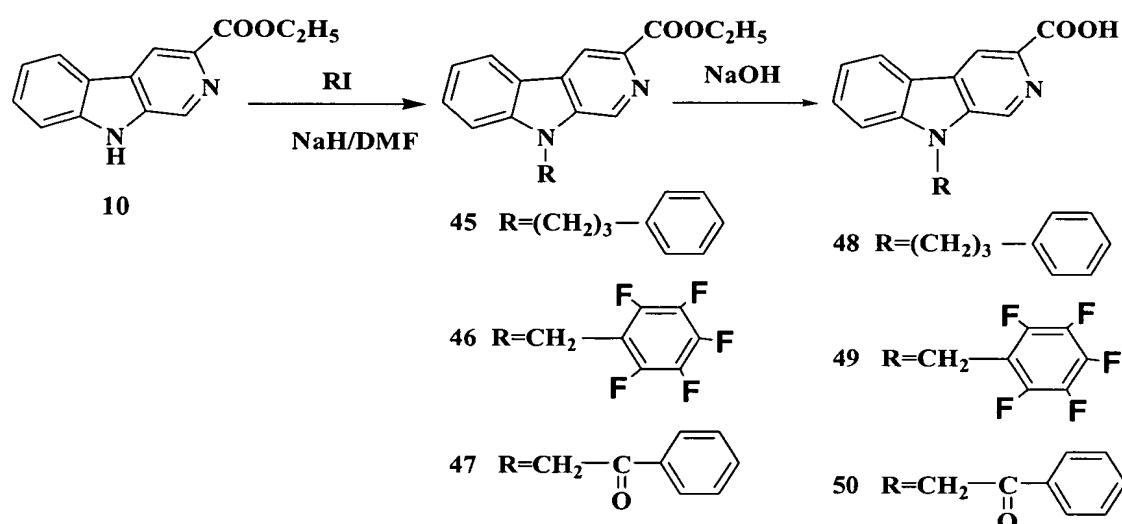
Example 70

Synthesis of ethyl 9-phenylpropyl-β-carboline-3-carboxylate (45)

Compound 10 (1.2g, 5mmol) was mixed with DMF (30ml). After stirring the mixture at room temperature for 15 minutes, NaH (0.6g, 15mmol) was added and stirred until the solution became clear. After that, 1-bromine-3-phenylpropane (2ml) was added. The mixture was refluxed for 4 h. THF was evaporated in reduced pressure. The resulting solution was added 200 ml 2N HCl solution. The mixture was extracted with ethyl ether. The aqueous phase was neutralized by saturated $NaHCO_3$ solution and then extracted with ethyl acetate. The organic phases were combined, washed with water and brine, dried over sodium sulfate, filtered, concentrated in vacuum and purified with silica gel column chromatography with ethyl acetate as the

eluent. Upon recrystallization, white needle crystals (1.0g, 56%) were obtained, mp 140-142℃.

Example 71

Synthesis of ethyl 9-(2',3',4',5',6'-pentafluoro)benzyl-β-carboline-3-carboxylate (46)

Compound 10 (1.2g, 5mmol) was mixed with DMF (30ml). After stirring the mixture at room temperature for 15 minutes, NaH (0.6g, 15mmol) was added and stirred until the solution became clear. After that, α-bromine-2,3,4,5,6-pentafuorobenzyl (1ml) was added. The mixture was stirred at room temperature for 1 hour. The subsequent steps were conducted according to those for synthesizing compound 45 to afford white crystals (1.3g, 62%), mp 153-154℃.

Example 72

Synthesis of ethyl 9-acetophenone-β-carboline-3-carboxylate (47)

Compound 10 (1.2g, 5mmol) was mixed with DMF (30ml). After stirring the mixture at room temperature for 15 minutes, NaH (0.6g, 15mmol) was added and stirred until the solution became clear. After that, 2-bromine-acetyl benzophenone (2.0g) was added. The mixture was refluxed for 4 h. The subsequent steps were conducted according to those for synthesizing compound 45 to afford white crystals (0.9g, 50%), and mp 246-248℃.

Example 73

General procedure for the preparation of 9-substituted-β-carboline-3-carboxylic acid

9-Substituted-β-carboline-3-carboxylate (10 mmol), NaOH (50 mmol), water (100ml) and ethanol (50ml) were mixed. The mixture was refluxed for 2 h. The reaction mixture was cooled to room temperature, and the pH was adjusted to 6 with 5M HCl. After cooling, filtration, wash with water, drying, light yellow solids were obtained. Examples 74 to 76 were treated according to the above operational steps.

Example 74

Synthesis of 9-phenylpropyl-β-carboline-3-carboxylic acid (48): light yellow solids were obtained, the yield was 97%, and mp 213-215℃.

Example 75

Synthesis of 9-(2',3',4',5',6'-pentafluoro)benzyl-β-carboline -3-carboxylic acid (49): white solids were obtained, the yield was 98%, and mp > 270℃.

Example 76

Synthesis of 9-acetophenone-β-carboline-3-carboxylic acid (50): light yellow solids (1.6g, 97%) were obtained, mp > 270℃.

Scheme VII

31 R$_1$=CH$_2$CH$_3$
33 R$_1$=CH$_2$C$_6$H$_5$

51 R$_1$=CH$_2$CH$_3$
52 R$_1$=CH$_2$C$_6$H$_5$

53a R$_1$=CH$_2$CH$_3$
55b R$_1$=CH$_2$C$_6$H$_5$

53 R$_1$=CH$_2$CH$_3$ R$_2$=CH$_3$
54 R$_1$=CH$_2$CH$_3$ R$_2$=CH$_2$CH$_3$
55 R$_1$=CH$_2$C$_6$H$_5$ R$_2$=CH$_2$CH$_3$
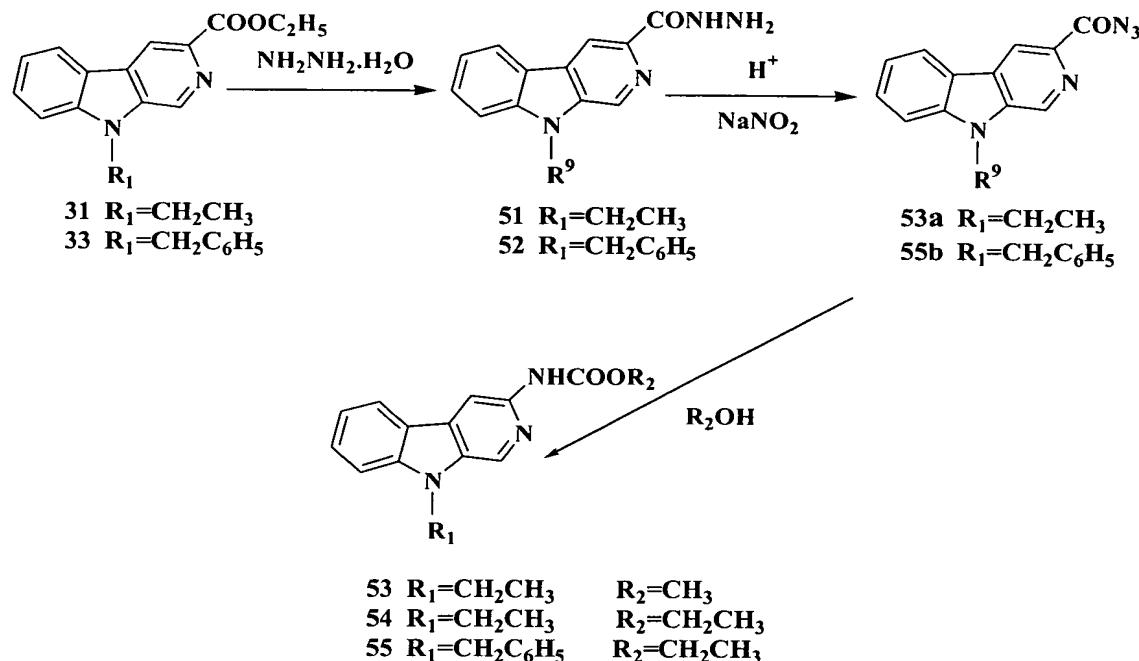
Example 77

Synthesis of 3-carbohydrazine 9-ethyl-β-carboline (51)

Compound 31 (2.7g, 10mmol) was dissolved into ethanol (50ml). 85% Hydrazine hydrate (15ml) was added. The mixture was refluxed by heating for 6 h and concentrated to 30 ml in reduced pressure. After cooling, filtration, wash with ethanol, and natural drying in the air, white solids (2.0g, 83%) were obtained. Samples for analysis could be recrystallized by 90% ethanol to obtain white flaring

sheet-like crystals, mp 195-196℃.

Example 78

Synthesis of 3-(azidocarbonyl)-9-ethyl-β-carboline (53a)

Concentrated HCl (20ml) was added dropwise into a mixed suspension formed from compound 51 (2.54g, 10mmol) and water (200ml). The light yellow solution was cooled in an ice bath to 0℃, and then an aqueous solution (30ml) of nitrous acid (2.1g, 30mmol) was added dropwise to react with the light yellow solution at 0℃ for 30 minutes. The mixed reaction solution was then alkalified with a saturated NaHCO₃ solution. Solids were collected by filtration. After being washed by water and vacuumly dried, yellow solids (2.3g) were obtained. The solids were apt to be decomposed and further purification was not necessary and used directly for the next steps.

Example 79

Synthesis of 9-ethyl-3[(methoxycarbonyl)amino]-β-carboline (53)

Compound 53a (0.6g, 5mmol) was dissolved into methanol (50ml). The mixture was refluxed for 10 h. The reaction liquid was cooled and concentrated to 30 ml in vacuum. After recrystallization with anhydrous ethanol, filtration, wash with a small amount of ethanol, white solids (0.4g, 59%) were obtained, mp 213-214℃.

Example 80

Synthesis of 3-[(ethoxycarbonyl)amino]- 9-ethyl-β-carboline (54)

Compound 53a (1.32g, 5mmol) was dissolved into ethanol (100ml). The mixture was refluxed for 10 h. The reaction liquid was cooled and concentrated to 30 ml in vacuum. After recrystallization and filtration, white solids (0.8g, 56%) were obtained, mp 217-218℃.

Example 81

Synthesis of 3-carbohydrazide-9-benzyl-β-carboline (52)

Compound 33 (3.3g, 10mmol) was dissolved into ethanol (100ml). 85% Hydrazine hydrate (20 ml) was added. The mixture was refluxed for 10 h and concentrated to 50 ml by decompression. After cooling,

filtration, wash with ethanol, and natural drying in the air, white solids (2.8g, 87%) were obtained, and mp 209-211℃.

Example 82

Synthesis of 3-(azidocarbonyl)-9-benzyl-β-carboline (55a)
Concentrated HCl (20 ml) was added dropwise into a mixed

suspension formed from compound 55a (3.16g, 10mmol) and water

(500ml). The light yellow solution was cooled in an ice bath to 0℃,

and then an aqueous solution (50ml) of nitrous acid (2.1g, 30mmol)

was added dropwise to react with the light yellow solution at 0℃ for

30 minutes. The mixed reaction solution was then alkalified with a

saturated $NaHCO_3$ solution. Solids were collected by filtration. After

being washed by water and vacuumly dried, yellow solids (2.9g) were

obtained with a tendency to decompose. The material was used

without further purification for the following steps.

Example 83

Synthesis of 3-[(ethoxycarbonyl)amino]-9-benzyl-β-carboline (55)

Compound 55a (2.9g, 8.86mmol) was dissolved into ethanol (150 ml). The mixture was refluxed 10 h. The reaction liquid was cooled and concentrated to 30 ml in reduced pressure. After recrystallization with ethyl acetate, white solids (1.8g, 57%) were obtained. Samples for analysis could be recrystallized by 90% ethanol, and mp 217-218℃.

Physico-chemical properties, TLC and spectra analyses of 3,9-disubstituted-β-carboline derivatives

Table 12 Physico-chemical data of 3,9-disubstituted β-carboline derivatives

Compd	Formula	FW	Yield	Appearance	Solubility	Mp (℃)

			(%)			
26	$C_{14}H_{12}N_2O_2$	240	75	white needle-like crystals	soluble in alcohols, ethers, esters, chloroform etc.	215-216
27	$C_{15}H_{14}N_2O_2$	254	79	white needle-like crystals	soluble in alcohols, ethers, esters, chloroform etc.	155-156
28	$C_{17}H_{18}N_2O_2$	282	82	white needle-like crystals	soluble in alcohols, ethers, esters, chloroform etc.	181-183
29	$C_{20}H_{16}N_2O_2$	316	73	white crystals	soluble in alcohols, ethers, esters, chloroform etc.	187-188
30	$C_{15}H_{14}N_2O_2$	254	75	white needle-like crystals	soluble in alcohols, ethers, esters, chloroform etc.	139-140
31	$C_{16}H_{16}N_2O_2$	268	67	white needle-like crystals	soluble in alcohols, ethers, esters, chloroform etc.	117-118
32	$C_{19}H_{22}N_2O_2$	296	78	white needle-like crystals	soluble in alcohols, ethers, esters, chloroform etc.	76-77
33	$C_{21}H_{18}N_2O_2$	330	70	white solids	soluble in alcohols, ethers, esters, chloroform etc.	126-127
34	$C_{13}H_{10}N_2O_2$	226	99	light yellow solids	soluble in alcohols and DMSO	267−269
35	$C_{14}H_{12}N_2O_2$	240	98	light yellow solids	soluble in alcohols and DMSO	201−202
36	$C_{16}H_{16}N_2O_2$	268	99	light yellow solids	soluble in alcohols and DMSO	182−184
37	$C_{19}H_{14}N_2O_2$	302	94	light yellow solids	soluble in DMSO	261−262
38	$C_{17}H_{18}N_2O_2$	282	70	white needle	soluble in	235−238

					crystals	alcohols, ethers, esters, chloroform etc.	
39	$C_{18}H_{20}N_2O_2$	296	65	white needle crystals	soluble in alcohols, ethers, esters, chloroform etc.	86-88
40	$C_{21}H_{24}N_2O_2$	324	74	white needle crystals	soluble in alcohols, ethers, esters, chloroform etc.	94－95
41	$C_{23}H_{22}N_2O_2$	358	67	white solids	soluble in alcohols, ethers, esters, chloroform etc.	105－106
42	$C_{19}H_{16}N_2O_2$	288	52	white solids	soluble in alcohols, ethers, chloroform etc.	120－122
43	$C_{21}H_{18}N_2O_2$	330	94	white needle crystals	soluble in alcohols, ethers, chloroform etc.	141－142
44	$C_{26}H_{20}N_2O_2$	392	57	white solids	soluble in alcohols, ethers, chloroform etc.	169－170
45	$C_{23}H_{22}N_2O_2$	358	56	white crystals	soluble in alcohols, ethers, chloroform etc.	140－142
46	$C_{21}F_5H_{13}N_2O_2$	420	62	white solids	soluble in alcohols, ethers, chloroform etc.	153－154
47	$C_{22}H_{18}N_2O_3$	358	50	white solids	soluble in alcohols, ethers, chloroform etc.	246-248
48	$C_{21}H_{18}N_2O_2$	330	97	light yellow solids	soluble in DMSO	213-215
49	$C_{19}F_5H_9N_2O_2$	392	98	white solids	soluble in DMSO	＞270
50	$C_{20}H_{14}N_2O_3$	330	97	light yellow solids	soluble in DMSO	＞270
51	$C_{14}H_{14}N_4O$	254	83	white flaring crystals	soluble in alcohols and DMSO	195-196
52	$C_{19}H_{16}N_4O$	316	87	white flaring crystals	soluble in alcohols and DMSO	209-211
53	$C_{15}H_{15}N_3O_2$	269	59	white solids	soluble in alcohols and	213-214

					esters	
54	$C_{16}H_{17}N_3O_2$	283	56	white solids	soluble in alcohols and esters	217-218
55	$C_{21}H_{19}N_3O_2$	345	57	white solids	soluble in alcohols and esters	218-219

Table 13　　FAB-MS, IR and UV data of 3,9-disubstituted
β-carboline derivatives

Compd	Formula	FAB-MS m/e(M+1)	IR (KBr, cm^{-1})	UV λ_{max} (nm)
26	$C_{14}H_{12}N_2O_2$	241	3386,3089,2548,2056,1730, 1631,1525,1497,1431,1376, 1282,1207,1115	358,343,307, 272,236,219
27	$C_{15}H_{14}N_2O_2$	255	3404,3358,2985, 1723,1632, 1523, 1439,1337,1258	358,345,306, 273,236,205
28	$C_{17}H_{18}N_2O_2$	283	3446,2958,2866, 1734,1624, 1584, 1551,1428,1361, 1245, 1102	358,344,306, 273,236,222
29	$C_{20}H_{16}N_2O_2$	317	3026,2945,1731,1622,1583, 1553,1424,1335,1242, 1105	356,341,304, 272,235,205
30	$C_{15}H_{14}N_2O_2$	255	3446,3397,2602,2015,1721, 1628,1587,1328,1207,1110, 1012	357,342,305, 272,236,219
31	$C_{16}H_{16}N_2O_2$	269	3413,2984,1717,1632,1521, 1448,1334,1257,1006	358,344,305, 273,236,221
32	$C_{19}H_{22}N_2O_2$	297	3437,2956,1731,1623,1552, 1465,1366,1210,1102,1024	358,343,307, 273,235,220
33	$C_{21}H_{18}N_2O_2$	331	3424,3027,2973,1723,1621, 1550,1466,1366,1214,1104	355,341,304, 272,234,204
34	$C_{13}H_{10}N_2O_2$	227	3406,2250-3250,1716,1630, 1599,1405,1314,1200	384,360,273, 241,217
35	$C_{14}H_{12}N_2O_2$	241	3418,2250,3250,1714,1631, 1588,1408,1336,1248,1198	387,359,347, 273,239,220
36	$C_{16}H_{16}N_2O_2$	269	3424,3062,2956,1690,1629, 1500,1467,1371,1296,1132	387,359,345, 272,238,221

37	$C_{19}H_{14}N_2O_2$	303	3409,3056,2946,1663,1624, 1586,1377,1225	355,342,267, 239
38	$C_{17}H_{18}N_2O_2$	283	3401,2956,2869,1726,1696, 1625,1584,1504,1463,1332, 1107	358,342,305, 272,236,219
39	$C_{18}H_{20}N_2O_2$	297	3423,3052,2963,,2401,1998,188 4,1722,1627,1587,1496, 1269,1121	359,345,306, 273,239,220
40	$C_{21}H_{24}N_2O_2$	325	3433,3061,3023,2956,2866 1728,1624,1551,1464,1358, 1212,1104	359,344,307, 273,238,220, 205
41	$C_{23}H_{22}N_2O_2$	359	3051,2959,2930,2869,1700, 1620,1582,1550,,1462,1361,129 8,1246,1107,1052	355,341,304, 273,235,205
42	$C_{19}H_{16}N_2O$	289	3170, 2938,1627,1559,1495, . 1467,1363,1264,1205,1048	361,347,291, 284,239,215
43	$C_{21}H_{18}N_2O_2$	331	3428,3027,2942,2886,1726 1622,1496,1452,1352,1245, 1026	360,346,291, 284,239,214
44	$C_{26}H_{20}N_2O_2$	393	3400,3064,3035,2935,2889, 1709,1623,1584,1497,1461, 1336,1244,1109	356,341,305, 273,236
45	$C_{23}H_{22}N_2O_2$	359	3425,3057,3026,2983,2933 2905,1724,1622,1550,1500,146 1,1367,1246,1107,1023	358,342,305, 273,236,217, 207
46	$C_{21}F_5H_{13}N_2 O_2$	421	3399,3065,2987,2904,1709, 1659,1626,1523,1502,1467, 1337,1296,1245,1104,1019	335,298,270, 265,236,217
47	$C_{22}H_{18}N_2O_3$	359	3420,3058,2979,2930,1721, 1692,1625,1586,1502,1468, 1339,1224,1107	354,339,300, 271,239
48	$C_{21}H_{18}N_2O_2$	331	3197,3149,3023,2934,1692, 1629,1590,1499	358,346,268, 239,218,210
49	$C_{19}F_5H_9N_2O _2$	393	3435,2900,1688,1632,1597, 1380,1230,982,756	351,338,261, 239,215
50	$C_{20}H_{14}N_2O_3$	331	3421,2986,1756,1713,1628, 1589,1495,1366,1132,1018	353,265,241
51	$C_{14}H_{14}N_4O$	ʼ255	3298,3202, 2955,1666,1627, 1590,1531,1497,1458,1331, 1261,1128	359,343,303, 272,238,221
52	$C_{19}H_{16}N_4O$	317	3349,3300,3201,3059, 1619, 1556,1496,1461,1336,1200	356,342,272, 238,207
53	$C_{15}H_{15}N_3O_2$	270	3435,3207,2984,1727,1630, 1595,1472,1282,1228,1079	376,365,296, 251,241,203

54	$C_{16}H_{17}N_3O_2$	284	3420,3202,2974,1721,1627, 1585,1532,1471,1281,1219	376,364,296, 240,202
55	$C_{21}H_{19}N_3O_2$	346	3248,3200,2981,1722,1629, 1590,1534,1467,1281,1217, 1063	373,362,295, 251

Table 14 ^{1}H-NMR data of 3,9-disubstituted β-carboline derivatives

Compd	^{1}H-NMR （δ, CDCl$_3$）
26	8.94(1H,s, Ar-H),8.88(1H,s,Ar-H),8.20-8.21(1H,d,J=7.5Hz,Ar-H),7.65-7.68 (1H,m, Ar-H),7.50-7.52(1H,d,J=8Hz,Ar-H),7.36-7.39(1H,m,J=8Hz,Ar-H),4.06(3H,s,-OCH$_3$),4.00(3 H,s, NCH$_3$)
27	8.90-9.00(2H,m,Ar-H),8.21-8.23(1H,d,J=8Hz,Ar-H),7.65-7.68(1H,m,Ar-H),7.52-7.54(1H,d, J=8Hz,Ar-H),7.36-7.39(1H,m,Ar-H),4.51(2H,s,NCH$_2$CH$_3$),4.07(3H,s,OCH$_3$), 1.52(3H,s,NCH$_2$CH$_3$)
28	8.94(1H,s,H-4),8.89(1H,s,H-1),8.19-8.21(1H,d,J=7Hz,H-8),7.62-7.65(1H,m,H-5), 7.50-7.52(1H,d,J=7.5Hz,H-6),7.34-7.37(1H,m,H-7),4.41-4.44(2H,m,CH$_2$CH$_2$CH$_2$ -CH$_3$),4.06(3H,s,OCH$_3$),1.87-1.93(2H,m,CH$_2$CH$_2$CH$_2$CH$_3$),1.35-1.42(2H,m,CH$_2$CH$_2$.-CH$_2$C H$_3$),0.93-0.96(3H,m,CH$_2$CH$_2$CH$_2$CH$_3$)
29	8.89-8.90(2H,d,J=4Hz,H-4,H-1),8.21-8.22(1H,d,J=8Hz,H-8),7.58-7.61(1H,m,H-5), 7.47-7.48(1H,d,J=8.5Hz,H-6),7.35-7.38(1H,m,H-7),7.24-7.28(3H,m,Ar-H),7.13-7.15 (2H,m,Ar-H),5.60(2H,s,CH$_2$-Ar),4.05(3H,s,OCH$_3$)
30	8.94(1H,s,H-4),8.86(1H,s,H-1),8.19-8.20(1H,d,J=7.5Hz,H-8),7.66-7.67(1H,m,H-5), 7.49-7.51(1H,d,J=8.5Hz,H-6),7.35-7.37(1H,m,H-7),4.52-4.56(2H,m,OCH$_2$CH$_3$),3.98 (3H,s,NCH$_3$),1.48-1.51(3H,s,OCH$_2$CH$_3$)
31	8.93(1H,s,H-4),8.86(1H,s,H-1),8.18-8.21(1H,d,J=8Hz,H-8),7.62-7.64(1H,m,H-5), 7.48-7.50(1H,d,J=8Hz,H-6),7.33-7.36(1H,m,H-7),4.42-4.56(4H,m,OCH$_2$CH$_3$,NCH$_2$CH$_3$),1.4 6-1.52(6H,m,OCH$_2$CH$_3$,NCH$_2$CH$_3$)
32	8.98(1H,s,H-4),8.89(1H,s,H-1),8.21-8.23(1H,d,J=8Hz,H-8),7.63-7.66(1H,m,H-5), 7.51-7.53(1H,d,J=8.5Hz,H-6),7.36-7.38(1H,m,H-7),4.52-4.56(2H,m,OCH$_2$CH$_3$),4.42-4.44(2 H,m,CH$_2$CH$_2$CH$_2$CH$_3$),1.88-1.94(2H,m,CH$_2$CH$_2$CH$_2$CH$_3$),1.49-1.52(3H,m, OCH$_2$CH$_3$),1.35-1.42(2H,m,CH$_2$CH$_2$CH$_2$CH$_3$),0.93-0.96(3H,m,CH$_2$CH$_2$CH$_2$CH$_3$)

33	8.91(2H,m,H-4,H-1),8.23-8.25(1H,d,J=8Hz,H-8),7.59-7.62(1H,m,H-5),7.49-7.50(1H,d,J=8Hz,H-6),7.36-7.39(1H,m,H-7),7.25-7.27(3H,s,Ar-H),7.14-7.16(2H,m,Ar-H),5.62(2H,s,CH$_2$-Ar),4.51-4.55(2H,m,-OCH$_2$CH$_3$),1.47-1.50(3H,m,OCH$_2$CH$_3$)
34	9.19(1H,s,H-4),9.12(1H,s,H-1),8.42-8.44(1H,d,J=8.0Hz,H-8),7.81-7.88(2H,m,H-5,H-6),7.50-7.53(1H,m,H-7),4.16(1H,s,N CH$_3$)
35	9.16(1H,s,H-4),8.97(1H,s,H-1),8.31-8.33(1H,d,J=8.0Hz,H-8),7.75-7.81(2H,m,H-5,H-6),7.43-7.46(1H,m,H-7),4.63-4.67(1H,s,NCH$_2$CH$_3$),1.46-1.52(1H,s,NCH$_2$CH$_3$)
36	9.14(1H,s,H-4),8.94(1H,s,H-1),8.42-8.44(1H,d,J=8.0Hz,H-8),7.77-7.79(1H,d,=8.5Hz,H-5),7.65-7.68(1H,m,H-6),7.34-7.37(1H,m,H-7),4.56-4.59(2H,m,NCH$_2$CH$_2$-CH$_2$CH$_3$),1.79-1.85(2H,m,NCH$_2$CH$_2$CH$_2$CH$_3$),1.28-1.32(2H,m,NCH$_2$CH$_2$CH$_2$CH$_3$),0.87-0.90(3H,m,,NCH$_2$CH$_2$CH$_2$CH$_3$)
37	9.15(1H,s,H-4),8.96(1H,s,H-1),8.44-8.45(1H,d,J=8.0Hz,H-8),7.79-7.80(1H,d,J=8.0Hz,H-5),7.63-7.66(1H,m,H-6),7.35-7.38(1H,m,H-7),7.22-7.31(5H,m,Ar-H),5.85(2H,s,NCH$_2$Ar)
38	8.94(1H,s,H-4),8.83(1H,s,H-1),8.18-8.20(1H,d,J=7.5Hz,H-8),7.63-7.66(1H,m,H-5),7.48-7.50(1H,d,J=8.5Hz,H-6),7.34-7.37(1H,m,H-7),4.46-4.49(2H,m,OCH$_2$CH$_2$CH$_2$CH$_3$),3.97(3H,s,NCH$_3$),1.84-1.90(2H,m,J=7Hz,OCH$_2$CH$_2$CH$_2$CH$_3$),1.48-1.56(2H,m,OCH$_2$CH$_2$CH$_2$CH$_3$),0.99-1.02(3H,m,OCH2CH2CH2CH3)
39	9.02(1H,s,H-4),8.86(1H,s,H-1),8.20-8.22(1H,d,J=8Hz,H-8),7.63-7.67(1H,m,H-5),7.51-7.53(1H,d,J=7.5Hz,H-6),7.35-7.38(1H,m,H-7),4.47-4.51(4H,m,OCH$_2$CH$_2$CH$_2$CH$_3$,NCH$_2$CH$_3$),1.84-1.90(2H,m,OCH$_2$CH$_2$CH$_2$CH$_3$),1.49-1.56(2H,m,OCH$_2$CH$_2$CH$_2$CH$_3$),0.99-1.02(3H,m,OCH$_2$CH$_2$CH$_2$CH$_3$)
40	8.95(1H,s,H-4),8.85(1H,s,H-1),8.19-8.20(1H,d,J=7Hz,H-8),7.60-7.64(1H,m,H-5),7.49-7.50(1H,d,J=8.5Hz,H-6),7.32-7.36(1H,m,H-7),4.47-4.49(2H,m,OCH$_2$CH$_2$CH$_2$CH$_3$),4.37-4.42(2H,m,NCH$_2$CH$_2$CH$_2$CH$_3$),1.84-1.92(4H,m,OCH$_2$CH$_2$CH$_2$CH$_3$,NCH$_2$CH$_2$CH$_2$CH$_3$),1.49-1.56(2H,m,OCH$_2$CH$_2$CH$_2$CH$_3$),1.34-1.40(2H,m,NCH$_2$CH$_2$CH$_2$CH$_3$),0.99-1.02(3H,m,OCH$_2$CH$_2$CH$_2$CH$_3$),0.92-0.95(3H,m,NCH$_2$CH$_2$CH$_2$CH$_3$)
41	8.89((1H,s,H-4),8.85(1H,s,H-1),8.19-8.20(1H,d,J=8Hz,H-8),7.56-7.59(1H,m,H-5),7.45-7.46(1H,d,J=8.5Hz,H-6),7.33-7.36(1H,m,H-7),7.22-7.26(3H,m,Ar-H),7.11-7.13(2H,m,Ar-H),5.56(2H,s,-CH$_2$-Ar),4.45-4.48(2H,m,-OCH$_2$CH$_2$CH$_2$CH$_3$),1.82-1.88(2H,m,-OCH$_2$CH$_2$CH$_2$CH$_3$),1.47-1.54(2H,m,OCH$_2$CH$_2$CH$_2$CH$_3$),0.98-1.01(3H,m,OCH$_2$CH$_2$CH$_2$CH$_3$)

42	8.73(1H,s,H-4),8.13-8.15(1H,d,J=8Hz,H-1),7.96(1H,s,H-8),7.54-7.58(1H,m,H-5), 7.41-7.43(1H,d,J=8.5Hz,H-6),7.28-7.31(1H,m,H-7),7.23-7.27(3H,m,Ar-H),7.11-7.12 (2H,m,Ar-H),5.51(2H,s,CH$_2$Ar),4.94(2H,s,CH$_2$OH),4.01(1H,s,CH$_2$OH)
43	9.01(1H,s,H-4),8.30-8.31(1H,d,J=8Hz,H-1),8.20(1H,s,H-8),7.73-7.75(1H,d,J=8.5Hz,H-5),7.5 8-7.61(1H,m,H-6),7.19-7.31(6H,m,H-7,Ar-H),5.76(2H,s,NCH$_2$Ar),5.28(2H, s,CH$_2$),2.11(3H,s,CH$_3$)
44	8.96(1H,s,H-4),8.91(1H,s,H-1),8.22-8.24(1H,m,H-8),7.60-7.64(1H,m,H-5),7.49-7.55 (3H,m,H-6,H-7,Ar-H),7.31-7.40(4H,m,Ar-H),7.25-7.29(3H,m,Ar-H),7.12-7.14(2H, m,Ar-H),5.63(2H,s,0CH$_2$Ar),5.52(2H,s,NCH$_2$Ar)
45	8.89(2H,m,H-4,H-1),8.20-8.22(1H,d,J=7.5Hz,H-8),7.61-7.64(1H,m,H-5),7.41-7.42 (1H,m,H-6),7.34-7.37(1H,m,H-7),7.26-7.30(2H,m,Ar-H),7.19-7.22(1H,m,Ar-H),7.13-7.15(2 H,m,Ar-H),4.52-4.57(2H,m,NCH$_2$CH$_2$CH$_2$Ar),4.41-4.44(2H,m,NCH$_2$CH$_2$CH$_2$Ar),2.70-2.73 (2H,m,OCH$_2$CH$_3$),2.24-2.30(2H,m,NCH$_2$CH$_2$CH$_2$Ar)1.49-1.52(3H,m,OCH$_2$CH$_3$)
46	9.07(1H,s,H-4),8.86(1H,s,H-1),8.19-8.21(1H,d,J=8Hz,H-8),7.65-7.68(1H,m,H-5), 7.59-7.61(1H,d,J=8.5Hz,H-6),7.38-7.41(1H,m,H-7),5.67(2H,s,CH$_2$-Ar),4.52-4.56 (2H,m,OCH$_2$CH$_3$),1.49-1.51(3H,m,OCH$_2$CH$_3$)
47	8.73(1H,s,H-4),8.68(1H,s,H-1),8.18-8.19(1H,d,J=7.5Hz,H-8),7.52-7.55(1H,m,H-5), 7.11-7.42(7H,m,H-6,H-7,Ar-H),5.51-5.55(2H,s,CH$_2$COAr),4.24-4.28(2H,m,OCH$_2$ CH$_3$),1.25-1.31(3H,m,OCH$_2$CH$_3$)
48	9.13(1H,s,H-4),9.00(1H,s,H-1),8.46-8.48(1H,d,J=7.5Hz,H-8),7.76-7.78(1H,d,J= 8.0Hz,H-5),7.69-7.72(1H,m,H-6),7.38-7.41(1H,m,H-7),7.21-7.26(2H,m,Ar-H),7.13- 7.17(3H,m,Ar-H),4.63-4.66(2H,m,NCH$_2$CH$_2$CH$_2$Ar),2.49-2.51(2H,m,NCH$_2$CH$_2$CH$_2$Ar),2.1 4-2.20(2H,m,NCH$_2$CH$_2$CH$_2$Ar)
49	8.11-8.13(1H,m,H-4),8.00-8.02(2H,m,H-1,H-8),7.69-7.73(1H,m,H-5),7.69-7.72(1H,m,H-6),7. 38-7.41(1H,m,H-7),7.21-7.26(2H,m,Ar-H),7.13-7.17(3H,m,Ar-H),4.63- 4.66(2H,m, NCH$_2$CH$_2$CH$_2$Ar),
50	9.28(1H,s,H-4),9.16(1H,s,H-1),8.57-8.58(1H,d,J=7.5Hz,H-8),8.16-8.18(2H,d,J= 8.5Hz,H-5,H-6),7.64-7.81(5H,m,H-7,Ar-H),7.43-7.46(1H,m,Ar-H),6.45(2H,s,NCH$_2$-COAr)
51	9.61(1H,s,NH),9.03(1H,s,H-4),8.82(1H,s,H-1),8.40-8.42(1H,d,J=7.5Hz,H-8),7.75- 7.76(1H,d,J=8.5Hz,H-5),7.64-7.67(1H,m,H-6),7.32-7.35(1H,m,H-7),4.58-4.62(2H,m,NCH$_2$C H$_3$),4.54(2H,s,NH$_2$),1.37-1.40(3H,m,NCH$_2$CH$_3$)

52	9.62(1H,s,NH),9.04(1H,s,H-4),8.85(1H,s,H-1),8.43-8.45(1H,d,J=8Hz,H-8),7.77-7.79 (1H,d,J=8.5Hz,H-5),7.61-7.65(1H,m,H-6),7.33-7.36 (1H,m,H-7),7.21-7.30 (5H,m, ArH), 5.83(2H,s,NCH₂Ar),4.53-4.54(2H,s,NH₂)
53	8.67(1H,s,H-4),8.55(1H,s,H-1),8.16-8.17(1H,d,J=8Hz,H-8),7.56-7.59(1H,m,H-5), 7.40-7.42(1H,d,J=8Hz,H-6),7.23-7.26(1H,m,H-7),4.37-4.41(2H,m,NCH₂CH₃),3.87 (3H,s,OCH₃)1.45-1.48(3H,m,NCH₂CH₃)
54	8.68(1H,s,H-4),8.59(1H,s,H-1),8.15-8.17(1H,d,J=8Hz,H-8),7.55-7.59(1H,m,H-5), 7.39-7.40(1H,d,J=8.5Hz,H-6),7.23-7.26(1H,m,H-7),4.32-4.38(4H,m,NCH₂CH₃, OCH₂CH₃),1.41-1.47(6H,m,NCH₂CH₃,OCH₂CH₃)
55	8.88(1H,s,H-4),8.78(1H,s,H-1),8.21-8.22(1H,d,J=7.5Hz,H-8),8.06-8.08(2H,d, J=8Hz,H-5,H-6),7.67-7.70(1H,m,H-7),7.54-7.60(3H,m,Ar-H),7.35-7.38(1H,m,Ar-H),7.31-7.32(1H,d,d=8Hz,Ar-H),5.79(2H,s,NCH₂-Ar),4.51-4.55(2H,m,OCH₂CH₃),1.47-1.50(3H,m, OCH₂CH₃)

Synthesis of 1,3,9-trisubstituted β-carboline derivatives

Experimental instruments and reagents

The experimental instruments and reagents are as described above.

Synthetic route and operational steps

Scheme I

$$\text{11} \xrightarrow[\text{NaH/DMF}]{\text{RI}} \text{56–61} \xrightarrow{\text{NaOH}} \text{62–67}$$

Structures with COOC₂H₅ / COOH on β-carboline, N-R substituted:

11

56 R=CH₃
57 R=CH₂CH₃
58 R=CH₂
59 R=(CH₂)₃—(phenyl)
60 R=CH₂—(pentafluorophenyl)
61 R=CH₂—C(=O)—(phenyl)

62 R=CH₃
63 R=CH₂CH₃
64 R=CH₂
65 R=(CH₂)₃—(phenyl)
66 R=CH₂—(pentafluorophenyl)
67 R=CH₂—C(=O)—(phenyl)
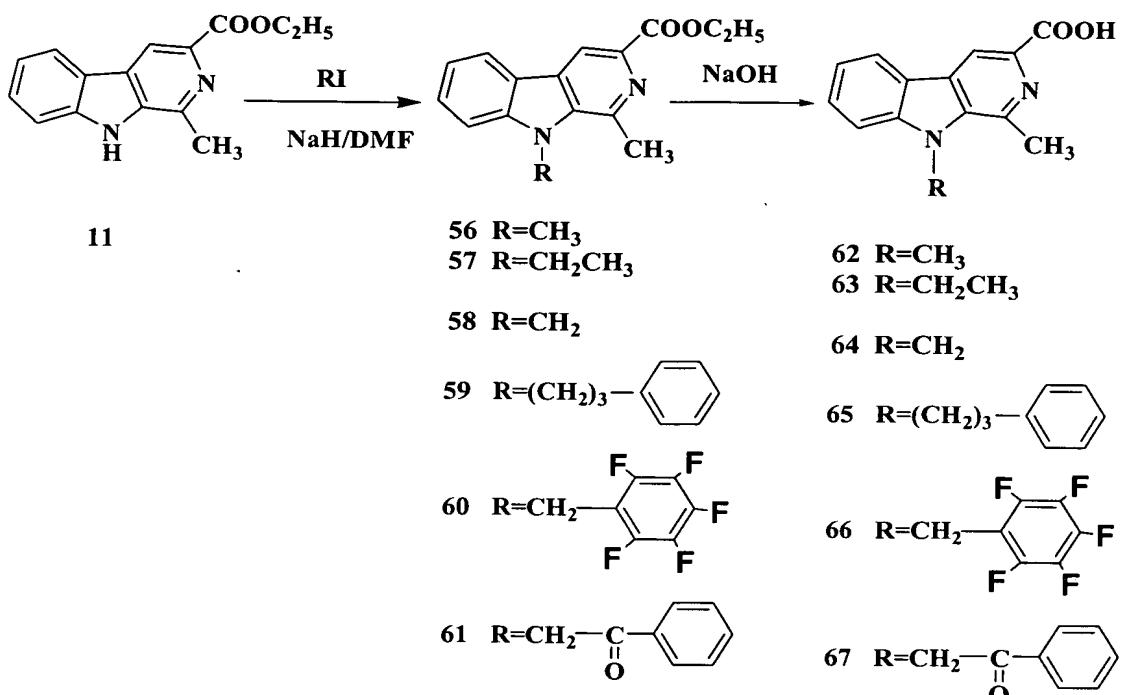
Example 84

General procedure for the preparation of 9-substituted-1-methyl-
β-carboline-3-carboxylates

Ethyl 1-methyl-β-carboline-3-carboxylate (10mmol) was mixed
with DMF (50ml) and stirred at room temperature until the solution
became clear. 60% NaH (50mmol) was added and stirred for 5
minutes. Alkene halide or aromatics (50mmol) was added. The
mixture was reacted at room temperature or refluxed. TLC track
measurement was conducted. After the reaction was finished, the
reaction mixture was poured into cold water, extracted with ethyl
acetate, washed with water and brine, dried, decolorized with
activated carbon, filtered and evaporated in reduced pressure, and
the residue was dissolved in 50 ml anhydrous ethanol. The pH was
adjusted to 3-4 with concentrated HCl. After concentration and
recrystallization with acetone/ethyl ether and filtration,
hydrochloride salts was formed. The hydrochloride salts were
added into a mixed solution of 100 ml water and ethyl acetate.
After being neutralized by sodium NaHCO₃, ethyl acetate layer was
isolated. The aqueous phase was extracted with ethyl acetate and
dried over anhydrous sodium sulfate, decolorized with activated
carbon, filtered, concentrated and purified with silica gel column
chromatography with ethyl acetate as the eluent.Upon
recrystallization, crystals was obtained. Examples 85 to 96 were
conducted according to the above operational steps.

Example 85

Synthesis of ethyl 1,9-dimethyl-β-carboline-3-carboxylate (56): white crystals (2.2g, 82%) were obtained, mp 141-142℃.

Example 86

Synthesis of ethyl 9-ethyl-1-methyl-β-carboline-3-carboxylate (57): white crystals (2.3g, 81%) were obtained, mp 96-98℃.

Example 87

Synthesis of ethyl 9-benzyl-1-methyl-β-carboline-3-carboxylate (58)

Compound 10 (2.54g, 10mmol) was mixed with DMF (50ml) and stirred at room temperature until the solution became clear. 60% NaH (1.2g) was added and stirred for 5 minutes. Benzyl bromide (6 ml) was added. TLC Track measurement with was conducted. The mixture was reacted at room temperature for 12 h. After the reaction was finished, the reaction mixture was poured into cold water, and extracted with ethyl acetate, washed with water and brine, dried, decolorized with activated carbon, evaporated. The residue was dissolved in 50 ml anhydrous ethanol. The pH of the solution was adjusted to 3-4 by introducing dry hydrogen chloride gas. After concentration, recrystallization with acetone/ethyl ether and filtration, hydrochloride salts was afforded, and 100ml water was added. After neutralizing by sodium bicarbonate, extracting by ethyl acetate, drying over anhydrous sodium sulfate, decolorizing with activated carbon, filtration, concentration, purifying with silica gel column chromatography with ethyl acetate as eluent. Upon recrystallization, white crystals (2.5g, 73%) was obtained, mp 155-156℃.

Example 88

Synthesis of ethyl 9-phenylpropyl-1-methyl-β-carboline-3-carboxylate (59)

Compound 10 (2.54g, 10mmol) was mixed with DMF (50ml) and stirred at room temperature until the solution became clear. 60% NaH (1.2g) was added and stirred for 5 minutes, followed by the addition of 1-bromine-3-phenyl propane (10ml), and TLC track measurement. The mixture was reacted at room temperature for about 15 h. The subsequent steps were conducted according to those for synthesizing compound 58. Finally, white crystals(2.8g, 75%) were obtained, mp 101-102°C.

Example 89

Synthesis of ethyl 9-(2',3',4',5',6'-pentafluoro)benzyl-1-methyl-β-carboline-3-carboxylate (60)

Compounds 10 (1.3g, 10mmol) was mixed with DMF (50ml), and was stirred at room temperature until the mixture became clear. The mixture was added 60% NaH (1.2g) and stirred for 5 minutes followed by the addition of α-bromine-2,3,4,5,6- pentafluorobenzyl (2 ml) and TLC track measurement. The mixture was reacted at room temperature for about 1 hour. The subsequent steps were conducted according to those for synthesizing compound 58 to afford white block crystals (1.5g, 68%), mp 145-146°C.

Example 90

Synthesis of ethyl 9-acetophenone-1-methyl-β-carboline-3-carboxylate (61): Afforded white solids (1.6g, 43%), mp was 219-220°C.

Example 91

Synthesis of ethyl 1-propyl-9-methyl-β-carboline-3-carboxylate (68): white crystals (2.2g, 74%) were obtained, mp 108-109°C.

Example 92

Synthesis of ethyl 1-propyl-9-ethyl-β-carboline-3-carboxylate (69): white crystals (2.0g, 65%) were obtained, mp 86-87°C.

Example 93

Synthesis of ethyl 9-benzyl-1-propyl-β-carboline-3-carboxylate (70): white crystals (1.8g, 64%) were obtained, mp 158-159°C.

Example 94

Synthesis of ethyl 9-phenylpropyl-1-propyl-β-carboline-3-carboxylate (71): white crystals (1.7g, 57%) were obtained, mp 92-93°C.

Example 95

Synthesis of methyl 1-phenyl-9-methyl-β-carboline-3-carboxylate (76): white crystals(2.4g, 76%)were obtained, mp 205-207°C.

Example 96

Synthesis of methyl 1-phenyl-9-ethyl-β-carboline-3-carboxylate (77): white crystals (2.3g, 69%) were obtained, mp 169-170°C.

Scheme II

Compound **13** → (RI, NaH/DMF) → **68** R=CH$_3$, **69** R=CH$_2$CH$_3$, **70** R=CH$_2$–C$_6$H$_5$, **71** R=(CH$_2$)$_3$–C$_6$H$_5$ → (NaOH) → **72** R=CH$_3$, **73** R=CH$_2$CH$_3$, **74** R=CH$_2$–C$_6$H$_5$, **75** R=(CH$_2$)$_3$–C$_6$H$_5$
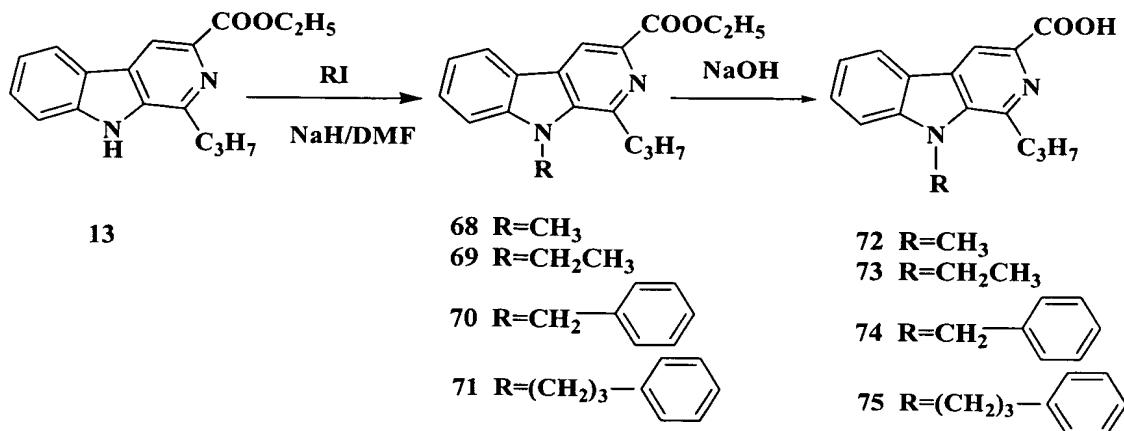
Example 97

General procedure for the preparation of 1,9-substituted-β-carboline-3-carboxylic acids

1,9-Disubstituted-β-carboline-3-carboxylate (10mmol), water (100 ml), ethanol (50ml) and NaOH (50mmol) were mixed and refluxed by heating for 2 h. And the ethanol was removed under reduced pressure. The mixture was neutralized (pH 5) with 5M HCl and cooled. The precipitate was collected, washed well with water and dried. Examples 98 to 109 were conducted according to the operational steps as mentioned above.

Example 98

Synthesis of 1,9-dimethyl-β-carboline-3-carboxylic acid (62): yellow solids (0.58g, 97%) were obtained, mp 262-264℃.

Example 99

Synthesis of 9-ethyl-1-methyl-β-carboline-3-carboxylic acid (63): yellow solids (0.62g, 97%) were obtained, mp 243-245℃.

Example 100

Synthesis of 9-benzyl-1-methyl-β-carboline-3-carboxylic acid (64):

yellow solids (0.78g, 98%) were obtained, mp 246-248℃.

Example 101

Synthesis of 9-phenylpropyl-1-methyl-β-carboline-3-carboxylic acid (65): yellow solids (0.84g, 97%)were obtained, mp 186-188℃.

Example 102

Synthesis of 9-(2',3',4',5',6'-pentafluoro)benzyl-1-methyl-β-carboline -3-carboxylic acid (66): gray solids (1.0g, 98%) were obtained, mp 191-193℃.

Scheme III

| 14 | 76 R=CH₃ | 78 R=CH₃ |
| | 77 R=CH₂CH₃ | 79 R=CH₂CH₃ |
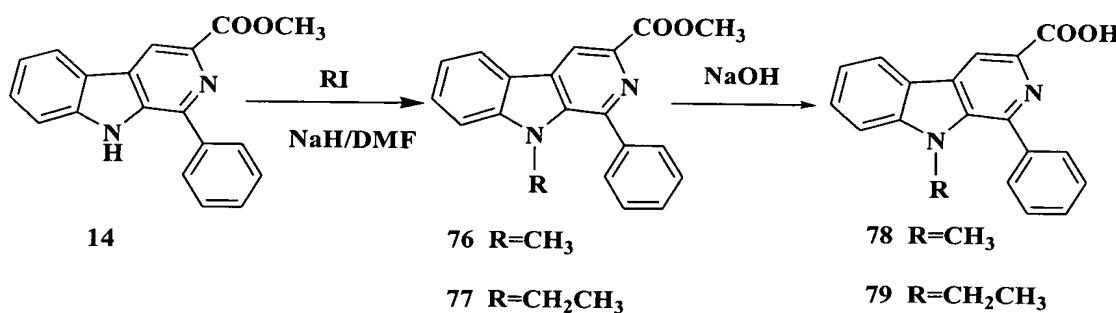
Example 103

Synthesis of 9-acetophenone-1-methyl-β-carboline-3-carboxylic acid (67): white solids (0.84g, 98%) were obtained, mp >270℃.

Example 104

Synthesis of 9-methyl-1-propyl-β-carboline-3-carboxylic acid (72): yellow solids (0.52g, 97%) were obtained, mp 181-183℃.

Example 105

Synthesis of 1-propyl-9-ethyl-β-carboline-3-carboxylic acid (73): yellow solids (0.54g, 96%) were obtained, mp 189-191℃.

Example 106

Synthesis of 9-benzyl-1-propyl-β-carboline-3-carboxylic acid (74): yellow solids (0.66g, 96%) were obtained, mp 193-194℃.

Example 107

Synthesis of 9-phenylpropyl-1-propyl-β-carboline-3-carboxylic acid (75): yellow solids (0.72g, 97%) were obtained, mp 223-224℃.

Example 108

Synthesis of 9-methyl-1-phenyl-β-carboline-3-carboxylic acid (78): Aforded light yellow solids (0.74g, 98%), mp 223-224℃.

Example 109

Synthesis of 9-ethyl-1-phenyl-β-carboline-3-carboxylic acid (79): Afforded light yellow solids (0.77g, 97%), mp 194-195℃.

Physico-chemical properties of, TLC and spectra analyses of 1,3,9-trisubstituted β-carboline derivatives

Table 15 Physico-chemical data of 1,3,9-substituted β-carboline derivatives

Compd	Formula	FW	Yield (%)	Appearance	Solubility	Mp (℃)
56	$C_{16}H_{16}N_2O_2$	268	82	white crystals	soluble in alcohols, ethers, chloroform etc.	141-142
57	$C_{17}H_{18}N_2O_2$	282	81	white crystals	soluble in alcohols, ethers, chloroform etc.	96-98
58	$C_{22}H_{20}N_2O_2$	344	73	white crystals	soluble in alcohols, ethers, chloroform etc.	155-156
59	$C_{24}H_{24}N_2O_2$	372	75	white crystals	soluble in alcohols, ethers, chloroform etc.	101-102

60	$C_{22}F_5H_{15}N_2O_2$	434	68	ash gray crystals	soluble in alcohols, ethers, chloroform etc.	145-146
61	$C_{23}H_{20}N_2O_3$	372	43	white flocculent solids	soluble in alcohols, ethers, esters, chloroform etc.	219-220
62	$C_{14}H_{12}N_2O_2$	240	97	light yellow solids	soluble in alcohols and DMSO	262-264
63	$C_{15}H_{14}N_2O_2$	254	97	light yellow solids	soluble in alcohols and DMSO	243-245
64	$C_{20}H_{16}N_2O_2$	316	98	light yellow solids	soluble in DMSO	246-248
65	$C_{22}H_{20}N_2O_2$	344	97	light yellow solids	soluble in DMSO	186-188
66	$C_{20}F_5H_{11}N_2O_2$	406	97	ash gray solids	soluble in DMSO	191-193
67	$C_{21}H_{16}N_2O_3$	344	98	white solids	soluble in DMSO	>270
68	$C_{18}H_{20}N_2O_2$	296	74	white crystals	soluble in alcohols, ethers, esters, chloroform etc.	119-110
69	$C_{19}H_{22}N_2O_2$	310	65	white crystals	soluble in alcohols, ethers, esters, chloroform etc.	86-87
70	$C_{24}H_{24}N_2O_2$	372	64	white crystals	soluble in alcohols, ethers, esters, chloroform etc.	158-159
71	$C_{26}H_{28}N_2O_2$	400	57	white crystals	soluble in alcohols, ethers and esters	92-93
72	$C_{16}H_{16}N_2O_2$	268	97	light yellow solids	soluble in alcohols and DMSO	181-183
73	$C_{17}H_{18}N_2O_2$	282	96	light yellow solids	soluble in alcohols and DMSO	189—191
74	$C_{22}H_{19}N_2O_2$	344	96	light yellow solids	soluble in DMSO	193-194
75	$C_{24}H_{23}N_2O_2$	372	97	light yellow solids	soluble in DMSO	176-178
76	$C_{20}H_{16}N_2O_2$	316	76	white crystals	soluble in alcohols, ethers, esters, chloroform etc.	205-207
77	$C_{21}H_{18}N_2O_2$	330	69	white crystals	soluble in alcohols, ethers,	169-170

					esters, chloroform etc.	
78	$C_{19}H_{14}N_2O_2$	302	98	white solids	soluble in DMSO	223-234
79	$C_{20}H_{16}N_2O_2$	316	97	white solids	soluble in DMSO	194-195

Table 16　FAB-MS, IR and UV data of 1,3,9-trisubstituted β-carboline derivatives

Compd	Formula	FAB-MS m/e(M+1)	IR (KBr，cm^{-1})	UV ($\lambda_{max,}$ nm)
56	$C_{16}H_{16}N_2O_2$	269	3044,2978,2903,1713,1620 1557,1456,1369,1249,1215 1138,1030	354,338,307, 272,239
57	$C_{17}H_{18}N_2O_2$	283	3359,3057,2974,2931,2902, 1687,1620,1556,1449,1368, 1342,1275,1243,1133,1026	354,338,307, 273,240
58	$C_{22}H_{20}N_2O_2$	345	3441,3059,2967,2929,1694, 1622,1561,1455,1342,1272, 1238,1136,1029	351,337,305, 272,239
59	$C_{24}H_{24}N_2O_2$	373	3059,2977,2930,2852,1694, 1620,1557,1454,1366,1341, 1257,1135,1028	355,339,308,273, 240,220
60	$C_{22}F_5H_{15}N_2O_2$	435	3398,2974,2931,1708,1629 1503,1454,1341,1268,1122, 1033	346,332,301, 271,265,238
61	$C_{23}H_{20}N_2O_3$	373	3063,3041,2998,1665,1595 1447,1330,1219,1018,711	250
62	$C_{14}H_{12}N_2O_2$	241	3558,3332,2250-3250,1936, 1712,1621,1344,1215,1050	355,340,269 239
63	$C_{15}H_{14}N_2O_2$	255	3393,2250-3250,1714,1621, 1589,1366,1233,1130,1053	355,270,240, 223
64	$C_{20}H_{16}N_2O_2$	317	2250-3750,1720,1615,1340,	352,338,268

			1206	239
65	$C_{22}H_{20}N_2O_2$	345	3417,2500-3250,1740,1624, 1591,1355,1061	356,269,240
66	$C_{20}F_5H_{11}N_2O_2$	407	3422,2250-3250,1754,1652, 1625,1592,1491,1360,1131, 1017	349,336,268, 238
67	$C_{21}H_{16}N_2O_3$	345	2250-3500,1667,1626,1370, 1104	345,334,265 239,216
68	$C_{18}H_{20}N_2O_2$	297	2957,2868,1703,1555,1466, 1367,1263,1135,1053,739	354,339,307, 272,240
69	$C_{19}H_{22}N_2O_2$	311	3052,2960,2868,1694,1555, 1446,1344,1243,1132	355,340,307, 273,240
70	$C_{24}H_{24}N_2O_2$	373	3430,2960,2934,2868,1727, 1619,1556,1462,1339,1259, 1223,1147,1052	352,337,304, 272,240
71	$C_{26}H_{28}N_2O_2$	401	3068,2994,2968,2929,2870, 1701,1620,1556,1450,1365, 1258,1132,1058	355,339,307, 273,241,201
72	$C_{16}H_{16}N_2O_2$	269	3142,3059,2960,2869,1739, 1620,1582,1470,1359,1248, 1128	356,342,269, 240
73	$C_{17}H_{18}N_2O_2$	283	3191,2965,2871,1745,1619, 1558,1456,1354,1228,1120	356,342,268, 240
74	$C_{22}H_{19}N_2O_2$	345	3064,2957,2923,2866,1752, 1643,1589,1456,1353,1209, 1130	353,339,268, 240
75	$C_{24}H_{23}N_2O_2$	373	3163,3068,2963,2933,1745, 1620,1583,1460,1362,1245, 1088	356,343,269, 240
76	$C_{20}H_{16}N_2O_2$	317	3426,3051,2944,1723,1622 1557,1493,1435,1356,1262,	360,346,309, 275,233

			1223,1129	
77	$C_{21}H_{18}N_2O_2$	331	3429,3054,2980,1724,1621, 1556,1429,1351,1247,1133, 1051	360,345,309, 275,234
78	$C_{19}H_{14}N_2O_2$	303	2000-3250,1754,1681,1622, 1557,1392,1262,1051	360,273,237
79	$C_{20}H_{16}N_2O_2$	317	3283,2974,1730,1620,1559, 1451,1355,1299	360,273,239

Table 17 ^{1}H-NMR data of 1,3,9-trisubstituted β-carboline derivatives

Compd	^{1}H-NMR（δ，DMSO)
56	8.71(1H,s,H-4),8.14-8.16(1H,d,J=8Hz,H-8),7.60-7.63(1H,m,H-5),7.45-7.47(1H,d, J=8.5Hz,H-6),7.31-7.34(1H,m,H-7),4.50-4.54(2H,m,OCH$_2$CH$_3$),4.16(3H,s,NCH$_3$),3.15(3H, s,CH$_3$),1.48-1.50(3H,m,OCH$_2$CH$_3$)
57	8.74(1H,s,H-4),8.16-8.18(1H,d,J=7.5Hz,H-8),7.60-7.63(1H,m,H-5),7.48-7.50(1H, d,J=8.5Hz,H-6),7.31-7.35(1H,m,H-7),4.62-4.66(2H,m,NCH$_2$CH$_3$),4.50-4.55(2H,m,OCH$_2$C H$_3$),3.13(3H,s,CH$_3$),1.46-1.50 (6H,m,NCH$_2$CH$_3$,OCH$_2$CH$_3$)
58	8.80(1H,s,H-4),8.22-8.23(1H,d,J=7.5Hz,H-8),7.55-7.58(1H,m,H-5),7.40-7.41(1H,d,J=8.5Hz ,H-6),7.34-7.37(1H,m,H-7),7.24-7.28(3H,m,Ar-H),6.94-6.96(2H,m,Ar-H),5.85(2H,s,NCH$_2$ Ar),4.50-4.54(2H,m,OCH$_2$CH$_3$),2.96(3H,s,CH$_3$),1.47-1.50(3H,m,OCH$_2$CH$_3$)
59	8.73(1H,s,H-4),8.15-8.17(1H,d,J=7.5Hz,H-8),7.56-7.59(1H,m,H-5),7.18-7.35(7H,m,H-6,H-7,Ar-H),4.49-4.58(4H,m,OCH$_2$CH$_3$,NCH$_2$CH$_2$CH$_2$Ar),2.97(3H,s,CH$_3$),2.74-2.77(2H,m, NCH$_2$CH$_2$CH$_2$Ar),2.14-2.20(2H,m,NCH$_2$CH$_2$CH$_2$Ar),1.47-1.50(3H,m,OCH$_2$CH$_3$)
60	8.75(1H,s,H-4),8.17-8.19(1H,d,J=8.5Hz,H-8),7.56-7.59(1H,m,H-5),7.34-7.38(2H,m,H-6, H-7),5.99(2H,s,NCH$_2$Ar),4.51-4.56(2H,m,OCH$_2$CH$_3$),3.16(3H,s,CH$_3$),1.48-1.51(3H,m, OCH$_2$CH$_3$)
62	8.89(1H,s,H-4),8.44-8.45(1H,d,J=8Hz,H-8),7.82-7.83(1H,d,J=8.5Hz,H-5),7.70-7.73(1H,m, H-6),7.37-7.40(1H,m,H-7),4.50-4.54(3H,s,NCH$_3$),3.18(3H,s,CH$_3$)
63	8.89(1H,s,H-4),8.45-8.46(1H,d,J=7.5Hz,H-8),7.83-7.85(1H,d,J=8.5Hz,H-5),7.70-7.73(1H,m ,H-6),7.37-7.41(1H,m,H-7),4.72-4.76(2H,m,NCH$_2$CH$_3$),3.14(3H,s,CH$_3$),1.40-1.42(3H,m,

	NCH$_2$CH$_3$)
64	8.80(1H,s,H-4),8.31-8.35(1H,d,J=7.5Hz,H-8),7.65-7.68(1H,d,J=8.0Hz,H-5),7.58-7.60(1H,m,H-6), 7.15-7.30 (6H,m,H-7,Ar-H), 5.78(2H,m,NCH$_2$Ar), 2.95(3H,s,CH$_3$)
65	8.75(1H,s,H-4),8.36-8.37(1H,d,J=7.5Hz,H-8),7.68-7.69(1H,d,J=8.0Hz,H-5),7.61-7.64(1H,m, H-6),7.18-7.34(6H,m,H-7,Ar-H),4.64-4.67(2H,m,NCH$_2$CH$_2$CH$_2$Ar), 2.90(3H,s,CH$_3$), 2.73-2.76 (2H,m, CH$_2$CH$_2$CH$_2$Ar),2.06-2.12 (2H,m,NCH$_2$CH$_2$CH$_2$Ar)
66	8.80(1H,s,H-4),8.40-8.42(1H,d,J=8.0Hz,H-8),7.62-7.64(2H,m,H-5,H-6),7.34-7.37(1H,m,H-7),6.10(2H,s,N CH$_2$Ar),3.01-3.02(3H,s,CH$_3$)
68	8.69(1H,s,H-4),8.14-8.16(1H,d,J=8Hz,H-8),7.59-7.62(1H,m,H-5),7.46-7.47(1H,d,J=8.5Hz, H-6),7.30-7.33(1H,m,H-7),4.49--4.53(2H,m,OCH$_2$CH$_3$),4.11(3H,s,NCH$_3$),3.36-3.39(2H,m, CH$_2$CH$_2$-CH$_3$),1.87-1.92(2H,m,CH$_2$CH$_2$CH$_3$),1.46-1.49(3H,m,OCH$_2$CH$_3$),1.09-1.12(3H,m, CH$_2$CH$_2$CH$_3$)
69	8.73(1H,s,H-4),8.18-8.19(1H,d,J=8Hz,H-8),7.60-7.63(1H,m,H-5),7.50-7.52(1H,d,J=8.5Hz, H-6),7.32-7.35(1H,m,H-7),4.49-4.62(4H,m,NCH$_2$CH$_3$,OCH$_2$CH$_3$),3.30-3.34(2H,m,CH$_2$-CH$_2$CH$_3$),1.87-1.95(2H,m,CH$_2$CH$_2$CH$_3$),1.45-1.49(6H,m,NCH$_2$CH$_3$,OCH$_2$CH$_3$),1.10-1.13 (3H,m,CH$_2$CH$_2$CH$_3$)
70	8.78(1H,s,H-4),8.22-8.24(1H,d,J=8Hz,H-8),7.55-7.58(1H,m,H-5),7.41-7.42(1H,d,J=8.5Hz, H-6),7.34-7.37(1H,m,H-7),7.23-7.30(3H,m,Ar-H),6.94-6.95(2H,m,Ar-H), 5.79(2H, s, NCH$_2$Ar),4.49-4.54(2H,m,OCH$_2$CH$_3$),3.12-3.16(2H,m,CH$_2$CH$_2$CH$_3$), 1.78-1.84(2H,m, CH$_2$CH$_2$CH$_3$), 1.46-1.52(3H,m,OCH$_2$CH$_3$),0.96-1.01(3H,m,CH$_2$CH$_2$CH$_3$)
71	8.71(1H,s,H-4),8.15-8.17(1H,d,J=8Hz,H-8),7.55-7.59(1H,m,H-5),7.34-7.36(1H,d,J=8.5Hz, H-6),7.29-7.32(3H,m,H-7,Ar-H),7.18-7.25(3H,m,Ar-H),4.46-4.53(4H,m,NCH$_2$CH$_2$CH$_2$Ar, OCH$_2$CH$_3$),3.15-3.18(2H,m,CH$_2$CH$_2$CH$_3$),2.74-2.77(2H,m,NCH$_2$CH$_2$CH$_2$Ar),2.11-2.11-(2 H,m,NCH$_2$CH$_2$CH$_2$Ar),1.77-1.84(2H,m,CH$_2$CH$_2$CH$_3$),1.46-1.49(3H,m,OCH$_2$CH$_3$),0.97-1.00(3H,m,CH$_2$CH$_2$CH$_3$)
72	8.76(1H,s,H-4),8.36-8.37(1H,d,J=7.5Hz,H-8),7.75-7.77(1H,d,J=8.0Hz,H-5),7.64-7.67(1H,m,H-6),7.32-7.35(1H,m,H-7),4.17(3H,s,NCH$_3$),3.35-3.38(2H,m,CH$_2$CH$_2$CH$_3$),1.85-1.89(2H,m,CH$_2$CH$_2$CH$_3$), 1.04-1.07(3H,m,CH$_2$CH$_2$CH$_3$)
73	8.77(1H,s,H-4),8.37-8.39(1H,d,J=7.5Hz,H-8),7.77-7.79(1H,d,J=8.5Hz,H-5),7.64-7.67(1H,m,H-6),7.32-7.36(1H,m,H-7),4.63-4.68(2H,m,NCH$_2$CH$_3$),3.26-3.30(2H,m,CH$_2$CH$_2$CH$_3$),1.86-1.93(2H,m,CH$_2$CH$_2$CH$_3$),1.37-1.40(3H,m,NCH$_2$CH$_3$),1.05-

	1.08(3H,m,CH$_2$CH$_2$CH$_3$)
74	8.83(1H,s,H-4),8.43-8.45(1H,d,J=8Hz,H-8),7.70-7.71(1H,d,J=8.0Hz,H-5),7.60-7.63(1H,m,H-6),7.35-7.38(1H,m,H-7),7.22-7.29(3H,m,Ar-H),6.93-6.95(2H,m,Ar-H),5.92(2H,s,NCH$_2$Ar),3.07-3.10(2H,m,CH$_2$CH$_2$CH$_3$),1.67-1.75(2H,m,CH$_2$CH$_2$CH$_3$),0.86-0.89(3H,m,CH$_2$CH$_2$CH$_3$)
75	8.75(1H,s,H-4),8.36-8.38(1H,d,J=7.5Hz,H-8),7.71-7.72(1H,d,J=8.0Hz,H-5),7.62-7.65(1H,m,H-6),7.19-7.35(6H,m,H-7,Ar-H),4.57-4.60(2H,s,NCH$_2$CH$_2$CH$_2$Ar),3.06-3.09(2H,m,CH$_2$CH$_2$CH$_3$),2.74-2.77(2H,m,NCH$_2$CH$_2$CH$_2$Ar),2.05-2.11(2H,m,CH$_2$CH$_2$CH$_3$),1.73-1.79(2H,m,NCH$_2$CH$_2$CH$_2$Ar),0.92-0.96(3H,m,CH$_2$CH$_2$CH$_3$)
76	8.91(1H,s,H-4),8.24-8.25(1H,d,J=7.5Hz,H-8),7.63-7.66 (3H,m,H-5,H-6,H-7),7.45-7.53(4H,m,Ar-H),7.37-7.40(1H,m,Ar-H), 4.03(3H,s,NCH$_3$),3.47(3H,s,OCH$_3$)
77	8.92(1H,s,H-4),8.23-8.26(1H,d,J=8Hz,H-8),7.60-7.64(3H,m,H-5,H-6,H-7),7.45-7.53(4H,m,Ar-H),7.35-7.39(1H,m,Ar-H),3.96-4.03(5H,s,NCH$_2$CH$_3$,OCH$_3$),0.98-1.01 (3H,m,,NCH$_2$CH$_3$)
78	8.93(1H,s,H-4),8.45-8.46(1H,d,J=7.5Hz,H-8),7.65-7.70(4H,m,H-5,H-6,H-7,Ar-H),7.56-7.60(3H,m,Ar-H),7.36-7.39(1H,m,Ar-H),3.44-3.47(3H,s,NCH$_3$)
79	8.98(1H,s,H-4),8.46-8.48(1H,d,J=8Hz,H-8),7.71-7.73(1H,d,J=8Hz,H-5),7.56-7.68(6H,m,H-6,H-7,Ar-H),7.36-7.39(1H,m,Ar-H),4.02-4.06(2H,m,NCH$_2$CH$_3$),0.86-0.89(3H,m,NCH$_2$CH$_3$)

Synthesis of 9-substituted β-carboline derivatives

Experimental instruments and reagents

Experimental instruments are as described above.

Chemical reagents

L-tryptophan (Acros Organic, U.S.), 40% formaldehyde solution (analytically pure, Guangzhou Chemical Reagent Plant), selenium

dioxide (Acros Organic, U.S.), benzyl bromide (chemically pure, Shanghai Chemical Reagent Co., China National Pharmaceutical Group), methyl iodide (analytically pure, Zhenjiang Yuhuan Biological Reagent Plant), ethyl iodide (analytically pure, Zhenjiang Yuhuan Biological Reagent Plant), iodo-*n*-butane (chemically pure, Shanghai Chemical Reagent Co., China National Pharmaceutical Group), and other domestically manufactured analytically pure or chemically pure reagents were used.

Scheme I

81 R=CH$_3$
82 R=C$_2$H$_5$
83 R=n-C$_4$H$_9$
84 R=CH$_2$C$_6$H$_5$
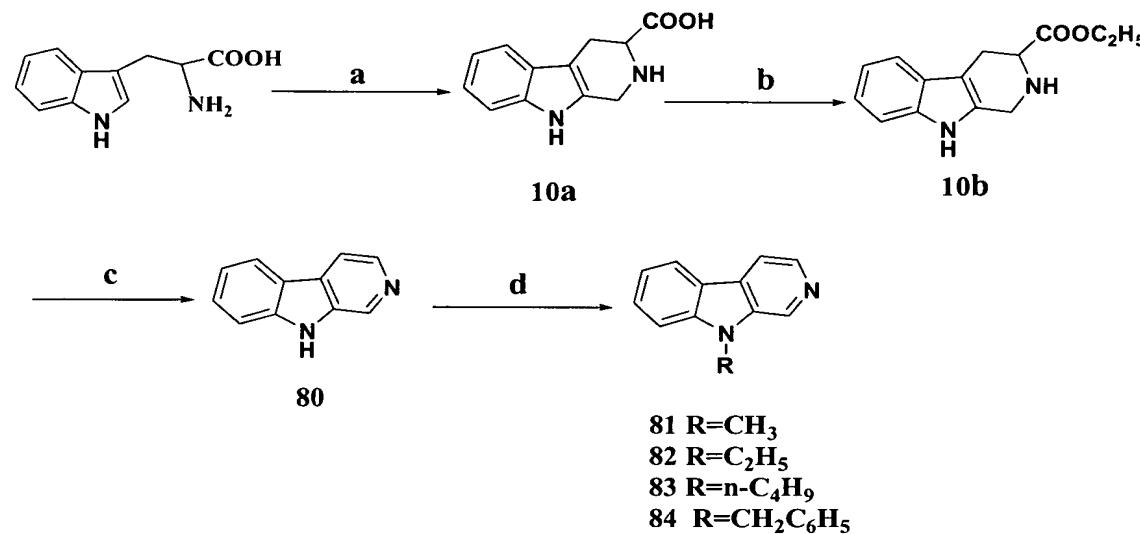
a) HCHO, NaOH, HCl; b) HAc, SeO$_2$; c)EtOH,SOCl$_2$, d)DMF, THF, NaH, RI

Example 110

Synthesis of *β*-carboline (80)

Compound 10b (24.4g, 0.1mol) was mixed with glacial acetic acid (500 ml) followed by the addition of selenium dioxide (20g, 0.2mol). The mixture was refluxed for 12 h. The glacial acetic acid was removed I . 1M NaOH solution (200ml) was added into the residues, then the mixture was extracted with ethyl acetate. The organic phases were combined, washed by 1M NaOH solution, water and brine, dried,

decolorized with activated carbon, filtered, evaporated and recrystallized with ethyl acetate to afford white solids (10.0g, 60%), and mp 197-198°C (reference[20]:198 to 200°C).

Example 111

General procedure for the preparation of 9-substituted β-carboline

β-carboline 80 (1.68 g, 10 mmol) was mixed with DMF (50ml) followed by the addition of alkyl halide or aromatics halide (50mmol). The mixture was stirred at room temperature for 5 h. TLC track measurement was conducted. After the reaction was finished, cold water was poured into the reaction mixture, and then extracted with ethyl acetate. The organic phases were combined, washed by water and brine, dried, decolorized with activated carbon, filtered and evaporated. The residues were purified by silica gel column chromatography with petroleum ether/acetone (2: 1) as the eluent.. The collected liquid was concentrated and recrystallized with ethyl acetate. Examples 112 to 115 were all treated according to the above procedures.

Example 112
Synthesis of 9-methyl-β-carboline (81): Afforded white needle solids (1.4g, 77%), and mp 108-109°C.

Example 113
Synthesis of 9-ethyl-β-carboline (82): Afforded yellow oil (1.5g, 76%).

Example 114
Synthesis of 9-n-butyl-β-carboline (83): Afforded white needle solids (1.6g, 71%), and mp 78-79°C.

Example 115

Synthesis of 9-Benzyl-β-carboline (84): Afforded white needle solids (1.8g, 69%), and mp 118-120℃.

Physico-chemical properties, TLC and spectra analyses of 9-substituted β-carboline derivatives

Table 18 physico-chemical data of 9-substituted β-carboline derivatives

Compd	Formula	FW	Yield (%)	Appearance	Solubility	Mp (℃)
80	$C_{11}H_8N_2$	168	60	white powder solids	soluble in alcohols, ethers, esters, chloroform etc.	198-200
81	$C_{12}H_{10}N_2$	182	65	white needle-like crystals	soluble in alcohols, ethers, esters, chloroform etc.	108-109
82	$C_{13}H_{12}N_2$	196	76	light yellow oil product	soluble in alcohols, ethers, esters, chloroform etc.	---
83	$C_{15}H_{16}N_2$	224	71	white needle-like crystals	soluble in alcohols, ethers, esters, chloroform etc.	78—79
84	$C_{18}H_{14}N_2$	258	69	white solids	soluble in alcohols, ethers, esters, chloroform etc.	118-120

Table 19 FAB-MS,IR and UV data of 9-substituted β-carboline derivatives

Compd	Formula	FAB-MS m/e(M+1)	IR (KBr, cm⁻¹)	UV λmax (nm)

80	$C_{11}H_8N_2$	169	ND	ND
81	$C_{12}H_{10}N_2$	183	2058,1638,1503,1335,1258, 835,754,718	360,346,289, 260,236,216
82	$C_{13}H_{12}N_2$	197	3043,2960,2484,2022,1633, 1500,1461,1355,1239,831, 746,719	365,290,260, 253,218,207
83	$C_{15}H_{16}N_2$	225	3013,2957,2525, 2030,1632, 1500,1458,1331,823,745, 720	362,347,290, 237,217
84	$C_{18}H_{14}N_2$	259	3023,2939,1619,1447,1332, 1200, 1025,821,752	358,344,289, 237,212

Table 20 ^{1}H-NMR data of 9-substituted β-carboline derivatives

Comp	^{1}H-NMR (δ, CDCl$_3$)
81	8.88(1H,s,H-4),8.46-8.47(1H,d,J=5Hz, H-1),8.13-8.14(1H,d,J=6Hz,H-8),7.94-7.95 (1H,d,J=5Hz, H-3),7.59-7.62(1H,m,H-5),7.45-7.46(1H,d, J=8.5Hz,H-6), 7.25-7.30 (1H,m,H-7),3.93(3H,s,CH$_3$)
82	8.84(1H,s,H-4),8.42-8.43(1H,d,J=5Hz, H-1),8.04-8.05(1H,d,J=8Hz,H-8),7.85-7.86 (1H,d,J=5Hz,H-3),7.50-7.53(1H,m,H-5),7.34-7.36(1H,d,J=8Hz,H-6),7.20-7.23(1H,m,H-7), 4.26-4.30(2H,m,CH$_2$CH$_3$),1.35-1.38(3H,m,CH$_2$CH$_3$)
83	8.86(1H,s,H-4),8.43-8.44(1H,d,J=5.5Hz,H-1),8.06-8.08(1H,d,J=8Hz,H-8),7.88- 7.89(1H,d,J=4.5Hz,H-3),7.52-7.55(1H,m,H-5),7.38-7.40(1H,d,J=8.5Hz,H-6),7.21- 7.25(1H,m,H-7),4.25-4.28(2H,m,CH$_2$CH$_2$CH$_2$CH$_3$),1.79-1.85(2H,m,CH$_2$CH$_2$CH$_2$ -CH$_3$),1.30-1.38(2H,m,CH$_2$CH$_2$CH$_2$CH$_3$),0.86-0.91(3H,m,CH$_2$CH$_2$CH$_2$CH$_3$)
84	8.84(1H,s,H-4),8.48(1H,s,H-1),8.15-8.16(1H,d,J=8Hz,H-8),7.98(1H,s,H-3),7.53-7.56 (1H,m,H-5),7.41-7.43(1H,d,J=8Hz,H-6),7.13-7.31(6H,m,J=8Hz,H-7,Ar-H),5.55(2H,s,CH$_2$ Ar)

Synthesis of 2,9-disubstituted β-carboline derivatives

Experimental instruments and reagents

The experimental instruments are as described above.

Chemical reagents

The chemical reagents are as described above.

Synthetic routes and operational steps

Scheme I

85 R=CH₂-⟨⟩ X=I

86 R=CH₂-⟨⟩ X=Br

87 R=CH₃

88 R=CH₂CH₃

89 R=CH₂-⟨⟩
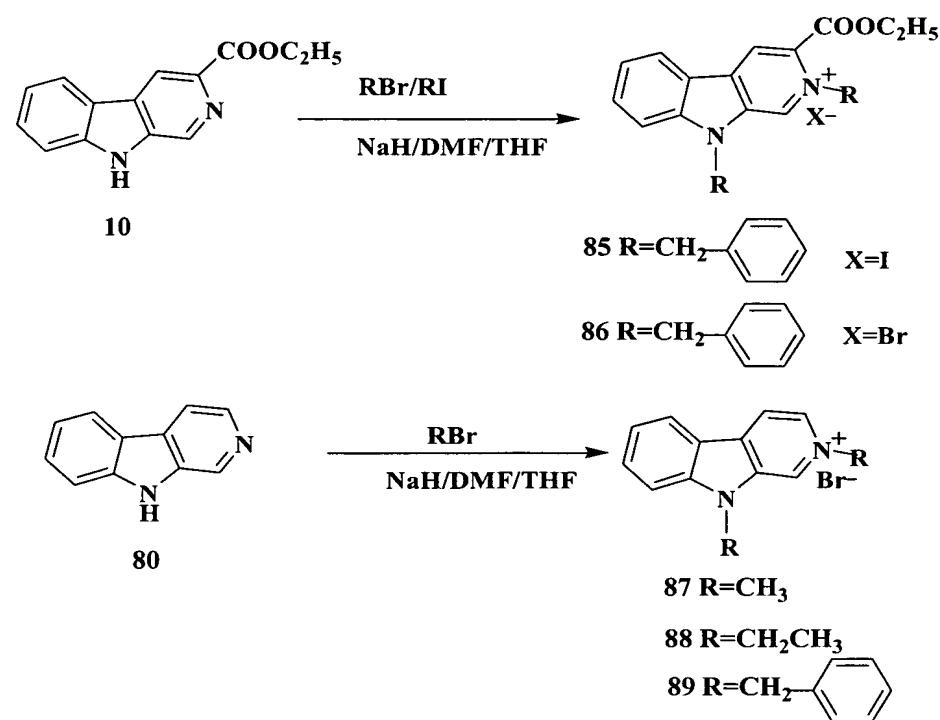
Example 116

Synthesis of 2,9-dibenzyl-3-ethoxycarbonyl-β-carbolinium iodate (85)

Compound 10 (1.2g, 5mmol) was mixed with DMF (50ml) and 60% NaH (0.6g, 15mmol). The mixture was stirred at room temperature for 5

minutes followed by the addition of benzyl iodide (5ml). The mixture was further stirred and reacted at 50 to 60°C for 2 h. The reaction mixture was poured into 50ml cold water and extracted by ethyl acetate. The organic phase was dried with anhydrous sodium sulfate and then concentrated to afford gold solids. The solids were recrystallized with anhydrous ethanol twice to afford gold solids (2.3g, 84%), mp >270°C.

Example 117

Synthesis of Synthesis of 2,9-dibenzyl-3-ethoxycarbonyl-β-carbolinium bromate (86)

Compound 10 (1.2g, 5mmol) was mixed with DMF (50ml) and 60% NaH (0.6g, 15mmol) followed by the addition of benzyl bromide (4ml). The mixture was stirred and reacted at 50 to 60°C for 5 h. The reaction mixture was poured into 50ml cold water and extracted by ethyl acetate. The organic phase was dried with anhydrous sodium sulfate and then concentrated to afford gold solids. The solids were recrystallized with anhydrous ethanol to afford gold solids (1.8g, 72%), mp >270°C.

Example 118

Synthesis of 2,9-dimethyl-β-carbolinium bromate (87)

Compound 80 (0.84g, 5mmol) was mixed with DMF (30ml) and 60% NaH (0.3g, 15mmol) followed by the addition of methyl bromide (30 mmol). The mixture was stirred and reacted at 50 to 70°C for 0.5 hour. The reaction mixture was poured into 100ml cold water and extracted by ethyl acetate. The extract was dried with anhydrous sodium sulfate and then concentrated to afford yellow solids. The

solids were recrystallized with anhydrous ethanol to afford white solids (1.8g, 73%), mp >270℃.

Example 119

Synthesis of 2,9-diethyl-β-carbolinium bromate (88)

Compound 80 (0.84g, 5mmol) was mixed with DMF (30ml) and 60% NaH (0.3g, 15mmol) followed by the addition of ethyl bromide (30 mmol). The mixture was stirred and reacted at 50 to 60℃ for 2 h. The reaction mixture was poured into 100ml cold water and extracted with ethyl acetate. The extract was dried with anhydrous sodium sulfate and then concentrated to afford yellow solids. The solids were recrystallized with anhydrous ethanol to afford yellow solids (1.1g, 63%), mp > 270℃.

Example 120

Synthesis of 2,9-dibenzyl-β-carbolinium bromate (89)

Compound 80 (0.84g, 5mmol) was mixed with DMF (30ml) and 60% NaH (0.3g, 15mmol). The mixture was stirred and reacted at room temperature for 10 minutes followed by the addition of benzyl bromide (50mmol). The mixture was stirred and reacted at 50 to 60℃ for 5 h. The reaction mixture was poured into 75ml cold water and extracted with ethyl acetate. The extract was dried with anhydrous sodium sulfate and then concentrated to afford light yellow solids. The solids were recrystallized with anhydrous ethanol to afford yellow solids (1.8g, 76%), mp > 270℃.

Physico-chemical constants, TLC and spectra analyses of 2,9-disubstituted β-carboline derivatives

Table 21 physico-chemical data of 2,9-disubstituted β-carboline derivatives

Compd	Formula	FW	Yield (%)	Appearance	Solubility	Mp(°C)
85	$C_{28}H_{25}N_2IO_2$	548	84	gold solids	soluble in alcohols, DMSO and water	>270
86	$C_{28}H_{25}N_2BrO_2$	501	72	gold solids	soluble in alcohols, DMSO and water	>270
87	$C_{13}H_{13}IN_2$	324	73	light yellow solids	soluble in methanol, DMSO and water	>270
88	$C_{15}H_{17}IN_2$	352	63	light yellow solids	soluble in methanol, DMSO and water	>270
89	$C_{25}H_{21}IN_2$	476	76	light yellow solids	soluble in methanol, DMSO and water	>270

Table 22 FAB-MS, IR and UV data of 2,9-disubstituted β-carboline derivatives

Compd	Formula	FAB-MS m/e(M+1)	IR (KBr, cm⁻¹)	UV λ_{max} (nm)
85	$C_{28}H_{25}N_2IO_2$	421	ND	ND
86	$C_{28}H_{25}N_2BrO_2$	421	3421,2976,1726,1630, 1517,1457,1367,1304, 1257,1096,1006	397,317,284, 243
87	$C_{13}H_{13}IN_2$	197	3447,2985,1807,1642, 1517,1467,1376,1335, 1262,1153	390,309,261,257, 220,210
88	$C_{15}H_{17}IN_2$	225	3437,2977,1815,1639, 1509,1458,1335,1243, 1158,1083	389,310,261, 257,220210
89	$C_{25}H_{21}IN_2$	349	3409,2982,2935,1644, 1511,1453,1337,1211, 1134	390, 313, 262, 235, 205

Table 23 ¹H-NMR data of 2,9-disubstituted β-carboline derivatives

Compd	¹H-NMR (δ, DMSO-d_6)
86	9.88(1H,s,H-4),9.30(1H,s,H-1),8.55-8.57(1H,d,J=7.5Hz,H-8),7.87-7.92(2H,m,H-5,

	H-6),7.55-7.59(1H,m,H-7),7.20-7.36(1OH,m,Ar-H),6.39(2H,s,$^+$N-CH$_2$-Ar),5.98(2H,s,NCH$_2$-Ar),4.44-4.48(2H,m,OCH$_2$CH$_3$),1.34-1.37(3H,m,OCH$_2$CH$_3$)
87	9.42(1H,s,H-4),8.66-8.67(1H,d,J=6.5Hz,H-1),8.51-8.52(1H,d,J=6.0Hz,H-8),8.43-8.45(1H,d,J=8Hz,H-3),7.83-7.91(2H,m,H-5,H-6),7.50-7.53(1H,m,H-7),4.57(3H,m,$^+$NCH$_3$),4.13(3H,m,NCH$_3$)
88	9.61(1H,s, H-4),8.68-8.69(1H,d,J=6.5Hz,H-1),8.61-8.63(1H,d,J=6.5Hz,H-8),8.42-8.44(1H,d,J=8Hz,H-3),7.84-7.89(2H,m,H-5,H-6),7.48-7.51(1H,m,H-7),4.83-4.87(2H,m,$^+$NCH$_2$CH$_3$),4.67-4.72(2H,m,NCH$_2$CH$_3$),1.75-1.78(3H,m, $^+$NCH$_2$CH$_3$),1.51-1.54 (3H,m,NCH$_2$CH$_3$)
89	9.57(1H,s, H-4),8.71(2H,s,H-1,H-8),8.42-8.44(1H,d,J=8.5Hz,H-3),7.81-7.82(2H,m, H-5, H-6),7.40-7.50(6H,m,H-7,Ar-H),7.19-7.28(5H,m,Ar-H),5.95(2H,s,N$^+$CH$_2$Ar), 5.86(2H,s, NCH$_2$Ar)

Example 121   Assay of acute toxicities

Materials and methods

1. Materials

(1) Chemicals: compounds 11, 16, 33, 36, 37, 42, 48, 55, 84 and 86

(2) Animals: Healthy mice, Kunming species (provided by Shanghai Experimental Animal Center, the Chinese Academy of Sciences, Certificate No.: Hudonghezhengzidi 107), weighing 19-20g, each group contained 10 mice (five males and five females)

(3) Solvents: physiological saline and 0.5% CMC-Na solution

2. Methods

(1) Dosage setting

Five grades of dosages for each sample according to the results of

the preliminary test, each dosage being 0.8 larger than the previous one

(2) Formulation of the medicament

During the experiment, each sample was added a small amount of Tween-80 to facilitate dissolution after the sample was weighed, and then 0.5% CMC-Na solution was gradually added to achieve the desired concentration. The experimental volume was 0.5 ml/ 20 g mouse.

(3) Administration manner
Single injection via intraperitoneal (i.p.) to different groups mice.

(4) Test on acute toxicity

Kunming mice were divided into different groups randomly according to their sex. The medicament was intraperitoneally administered to each group of mice according to the dosage setting. After the administration of the medicaments, mice were observed continuously for the first 2 h for any gross behavioral changes and deaths. Dead animals were dissected and examined for any possible organ damage. Survived animals were observed for another two weeks. Conditions of animals died within two week were recorded. After two weeks, all survived animals were sacrificed and checked macroscopically for possible damage to the heart, liver, kidneys and so on. Viscera that had substantive pathological changes were pathologically examined. According to the number of dead animals in each group, semi-lethal dosage (LD_{50} value) was calculated, and the maximum tolerance dosage (MTD) of compounds that had lower toxicity was calculated too.

Table 24　Acute toxic effect of compound 11 in mice
(administration via intraperitoneal)

Sex	Dosage (mg/kg)	Number of animals	Death distribution (day) 12345678910--21	Death rate	LD_{50}(95%CL) (mg/kg)
male	300	5	5000000000---0	100	
	240	5	4000000000---0	80	
	192	5	2000000000---0	40	
	153.6	5	1000000000---0	20	
	122.9	5	0000000000---0	0	
female	300	5	5000000000---0	100	
	240	5	5000000000---0	100	
	192	5	3000000000---0	60	
	153.6	5	2000000000---0	40	
	122.9	5	0000000000---0	0	
50% male	300	10	10000000000---0	100	183.47 (159.21-211.43)
50%	240	10	9000000000---0	90	

female	192	10	5000000000---0	50
	153.6	10	3000000000---0	30
	122.9	10	0000000000---0	0

Table 25　Acute toxic effect of compound 16 in mice

(administration via intraperitoneal)

Sex	Dosage (mg/kg)	Number of animals	Death distribution (day) 12345678910--21	Death rate	LD$_{50}$(95%CL) (mg/kg)	Sex
Male	300	5	1000000000---0	20	19.6/25.2	
	240	5	0000000000---0	0	19.7/25.7	
	192	5	0000000000---0	0	19.8/26.1	
	153.6	5	0000000000---0	0	19.8/26.4	
	122.9	5	0000000000---0	0	19.8/26.3	
female	300	5	2000000000---0	40	20.0/23.3	
	240	5	0000000000---0	0	20.2/24.2	
	192	5	0000000000---0	0	19.9/24.9	
	153.6	5	0000000000---0	0	20.1/24.7	
	122.9	5	0000000000---0	0	20.1/24.8	
50% male	300	10	3000000000---0	30		240
50% female	240	10	0000000000---0	0		
	192	10	0000000000---0	0		
	153.6	10	0000000000---0	0		
	122.9	10	0000000000---0	0		

Table 26 Acute toxic effect of compound 33 in mice
(administration via intraperitoneal)

Sex	Dosage (mg/kg)	Number of animals	Death distribution (day) 12345678910--21	Death rate	LD_{50}(95%CL) (mg/kg)
male	300	5	4000000000---0	80	
	240	5	2000000000---0	40	
	192	5	0000000000---0	0	
	153.6	5	0000000000---0	0	
	122.9	5	0000000000---0	0	
female	300	5	5000000000---0	100	
	240	5	3000000000---0	60	
	192	5	1000000000---0	20	
	153.6	5	0000000000---0	0	
	122.9	5	0000000000---0	0	
50% male	300	10	9000000000---0	90	240.38 (211.28-273.50)
50% female	240	10	5000000000---0	50	
	192	10	1000000000---0	10	
	153.6	10	0000000000---0	0	
	122.9	10	0000000000---0	0	

Table 27   Acute toxic effect of compound 36 in mice
(administration via intraperitoneal)

Sex	Dosage (mg/kg)	Number of animals	Death distribution (day) 12345678910--21	Death rate	LD$_{50}$(95%CL) (mg/kg)	Sex
male	300	5	1000000000---0	20	20.0/25.5	
	240	5	0000000000---0	0	20.2/25.0	
	192	5	0000000000---0	0	20.1/26.2	
	153.6	5	0000000000---0	0	20.1/26.2	
	122.9	5	0000000000---0	0	20.2/26.4	
female	300	5	2000000000---0	40	19.7/24.3	
	240	5	0000000000---0	0	19.5/24.5	
	192	5	0000000000---0	0	19.8/24.9	
	153.6	5	0000000000---0	0	19.7/24.8	
	122.9	5	0000000000---0	0	19.9/25.2	
50% male	300	10	3000000000---0	30		>300
50% female	240	10	0000000000---0	0		
	192	10	0000000000---0	0		
	153.6	10	0000000000---0	0		
	122.9	10	0000000000---0	0		

Table 28   Acute toxic effect of compound 37 in mice
(administration via intraperitoneal)

Sex	Dosage (mg/kg)	Number of animals	Death distribution (day) 12345678910--21	Death rate	LD$_{50}$(95%CL) (mg/kg)
male	300	5	5000000000---0	100	
	240	5	5000000000---0	100	
	192	5	3000000000---0	60	
	153.6	5	2000000000---0	40	
	122.9	5	0000000000---0	0	
female	300	5	5000000000---0	100	
	240	5	5000000000---0	100	
	192	5	4000000000---0	80	
	153.6	5	2000000000---0	40	
	122.9	5	1000000000---0	10	
50% male	300	10	10000000000---0	100	163.48 (141.56-188.76)
50 female	240	10	10000000000---0	100	
	192	10	7000000000---0	70	
	153.6	10	4000000000---0	40	
	122.9	10	1000000000---0	10	

Table 29   Acute toxic effect of compound 42 in mice
(administration via intraperitoneal)

Sex	Dosage (mg/kg)	Number of animals	Death distribution (day) 12345678910--21	Death rate	LD_{50}(95%CL) (mg/kg)
male	300	5	4000000000---0	80	
	240	5	2000000000---0	40	
	192	5	0000000000---0	0	
	153.6	5	0000000000---0	0	
	122.9	5	0000000000---0	0	
female	300	5	4000000000---0	80	
	240	5	3000000000---0	60	
	192	5	1000000000---0	20	
	153.6	5	0000000000---0	0	
	122.9	5	0000000000---0	0	
50% male	300	10	8000000000---0	80	247.13 (213.51-286.03)
50% female	240	10	5000000000---0	50	
	192	10	1000000000---0	10	
	153.6	10	0000000000---0	0	
	122.9	10	0000000000---0	0	

Table 30 Acute toxic effect of compound 48 in mice

(administration via intraperitoneal)

Sex	Dosage (mg/kg)	Number of animals	Death distribution (day) 12345678910--21	Death rate	LD$_{50}$(95%CL) (mg/kg)
male	300	5	5000000000---0	100	
	240	5	3000000000---0	60	
	192	5	1000000000---0	20	
	153.6	5	0000000000---0	0	
	122.9	5	0000000000---0	0	
female	300	5	5000000000---0	100	
	240	5	3000000000---0	60	
	192	5	2000000000---0	40	
	153.6	5	0000000000---0	0	
	122.9	5	0000000000---0	0	
50% male	300	10	10000000000---0	100	219.19 (193.25-248.61)
50% female	240	10	6000000000---0	60	
	192	10	3000000000---0	30	
	153.6	10	0000000000---0	0	
	122.9	10	0000000000---0	0	

Table 31   Acute toxic effect of compound 55 in mice

(administration via intraperitoneal)

Sex	Dosage (mg/kg)	Number of animals	Death distribution (day) 12345678910--21	Death rate	LD_{50}(95%CL) (mg/kg)	Sex
male	300	5	2000000000---0	40	20.1/25.3	
	240	5	0000000000---0	0	20.2/25.7	
	192	5	0000000000---0	0	20.0/25.9	
	153.6	5	0000000000---0	0	20.0/26.2	
	122.9	5	0000000000---0	0	20.0/26.1	
female	300	5	3000000000---0	60	19.5/24.0	
	240	5	0000000000---0	0	19.8/24.5	
	192	5	0000000000---0	0	19.7/24.7	
	153.6	5	0000000000---0	0	19.7/24.6	
	122.9	5	0000000000---0	0	19.9/24.9	
50% male	300	10	5000000000---0	50		240
50% female	240	10	0000000000---0	0		
	192	10	0000000000---0	0		
	153.6	10	0000000000---0	0		
	122.9	10	0000000000---0	0		

Table 32 Acute toxic effect of compound 84 in mice

(administration via intraperitoneal)

Sex	Dosage (mg/kg)	Number of animals	Death distribution (day) 12345678910--21	Death rate	LD$_{50}$(95%CL) (mg/kg)	Sex
male	300	5	1000000000---0	20	20.0/25.5	
	240	5	0000000000---0	0	20.2/25.1	
	192	5	0000000000---0	0	20.1/26.2	
	153.6	5	0000000000---0	0	20.0/26.3	
	122.9	5	0000000000---0	0	20.2/26.4	
female	300	5	2000000000---0	40	19.7/24.4	
	240	5	0000000000---0	0	19.6/24.6	
	192	5	0000000000---0	0	19.8/24.9	
	153.6	5	0000000000---0	0	19.8/24.7	
	122.9	5	0000000000---0	0	19.9/25.0	
50% male	300	10	3000000000---0	30		240
50% famle	240	10	0000000000---0	0		
	192	10	0000000000---0	0		
	153.6	10	0000000000---0	0		
	122.9	10	0000000000---0	0		

Table 33   Acute toxic effect of compound 86 in mice

(administration via intraperitoneal)

Sex	Dosage (mg/kg)	Number of animals	Death distribution (day) 12345678910--21	Death rate	LD$_{50}$(95%CL) (mg/kg)
male	100	5	1350000000---0	90	
	80	5	0132000000---0	60	
	64	5	0031100000---0	50	
	51.2	5	0011100000---0	20	
	40.96	5	0000000000---0	0	
female	100	5	2341000000---0	100	
	80	5	0322000000---0	70	
	64	5	0121000000---0	40	
	51.2	5	0111000000---0	30	
	40.96	5	0000000000---0	0	
50% male	100	10	3691000000---0	95	65.7 (58.25-74.11)
50% female	80	10	0454000000---0	65	
	64	10	0152100000---0	45	
	51.2	10	0122000000---0	25	
	40.96	10	0000000000---0	0	

3. Results

3.1 Observations of ordinary symptoms

After drug administration, all the mice exhibited symptoms such as quivering of abdominal muscles, stretch, loose hairs, and retardant action, but did not show obvious symptoms of nervous toxicity such as trembling, twists, and jumps. Death peak of the animal occurred the very day when the medicament was administered,

except for compound 86. As for compound 86, the death peak occurred 2 to 3 days after the medicament was administered. Generally, no obvious abnormal organs were observed after the dead animals were dissected. Survived animals recovered to normal state gradually.

3.2 Results of neurotoxicity and acute toxicity (LD_{50}/MTD)

See table 120-1 to table 120-10 for results of dosage-reaction value and LD_{50} value or MTD value.

For the convenience of comparison, the results [153] of the previous tests on acute toxicity and the results of the present tests are shown in table 34.

Table 34 Results of neurotoxicities and the acute toxicities (LD_{50}/MTD) of β-carboline derivatives
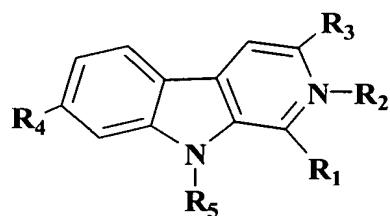
Compd	R_1	R_2	R_3	R_4	R_5	LD_{50}	MTD	Neurotoxic
1*	CH_3	H	H	CH_3O	H	59.00	—	++
2*	CH_3	H	H	CH_3O	CH_3	28.92	—	++
3*	CH_3	H	H	CH_3O	C_2H_5	24.25	—	++
4*	CH_3	H	H	CH_3O	n-C_4H_9	26.45	—	++
6*	CH_3	H	H	CH_3O	$CH_2C_6H_5$	147.82	—	++
11	CH_3	H	$CO_2C_2H_5$	H	H	183.47	—	–
16	C_6H_5-p-O H	H	CO_2CH_3	H	H	—	240	–

17*	H	H	COOH	H	H	135.22	—	—
26*	H	H	CO$_2$C$_2$H$_5$	H	CH$_3$	70.61	—	+
27*	H	H	CO$_2$C$_2$H$_5$	H	C$_2$H$_5$	95.06	—	+
33	H	H	CO$_2$C$_2$H$_5$	H	CH$_2$C$_6$H$_5$	240.38	—	—
36	H	H	COOH	H	n-C$_4$H$_9$	—	>300	—
37	H	H	COOH	H	CH$_2$C$_6$H$_5$	163.48	—	—
42	H	H	CH$_2$OH	H	CH$_2$C$_6$H$_5$	247.13	—	—
48	H	H	COOH	H	(CH$_2$)$_3$C$_6$H$_5$	219.19	—	—
55	H	H	NHCO$_2$C$_2$H$_5$	H	CH$_2$C$_6$H$_5$	—	240	—
84	H	H	H	H	CH$_2$C$_6$H$_5$	—	240	—
86	H	CH$_2$C$_6$H$_5$	CO$_2$C$_2$H$_5$	H	CH$_2$C$_6$H$_5$	65.7	—	—

Example 122 *In vitro* cytotoxicy assays

1. Materials and method

1.1 Materials
(1) Reagents
RPMI1640 culture medium (GIBCO, U.S.), MTT (Sigma, U.S.), Fetal Bovine Serum (GIBCO or Hyclone, U.S.), HEPES (Sigma, U.S.), trypsin operating fluid (0.125% trypsin, Sigma, 0.01%EDTA, dissolved in D-Hanks solution), DMSO (Sigma, U.S.), cell freezing solution (90%FBS+10%DMSO), D-Hanks balanced salt solution (i.e. Hanks solution free of calcium and magnesium ions : 8g NaCl, 0.4g KCl, 0.06g Na$_2$HPO$_4$.2H$_2$O, 0.06g KH$_2$PO$_4$.2H$_2$O, 0.35g NaHCO$_3$ were dissolved in tri-distilled water with no phenol red), PBS(8g NaCl, 0.2g KCl, 1.56g Na$_2$HPO$_4$.2H$_2$O, 0.2g KH$_2$PO$_4$.2H$_2$O were dissolved in tri-distilled water) were used. Chemicals: 88 compounds synthesized as described in the second part of the description and harmine of formula 1.

Cells: HepG2, PLA-801, Bel-7402, BGC-823, Hela, Lovo and NIH3T3.

(2) Instruments

A super clean bench, a CO_2 cell culture incubator, an universal microplate Reader (BIO-TEK INSTRUMENT, INC.), an inverted microscope, a liquid nitrogen tank, an ultrapure water system (Millipore, Inc), various models of filters, a positive air filtration system, a centrifuge, and a constant temperature water bath were used.

Other reagents and instruments used included penicillin sodium sulfate, streptomycin sulfate, DMSO (Sigma), sodium bicarbonate, alcohol, benzalkonium bromide solution, various models of cell culture bottles and dishes, 96 pore/24 pore/6 pore cell culture plates, glass centrifuge tubes, glass pipettes, suckers, cannula, cell counting plates, various models of pipettors, cell freezing tubes, forceps and the like.

1.2 Methods

(1) Culture solutions

Culture medium powder RPMI1640 was dissolved in a proper amount of tri-distilled water according to the instruction of the product. For every 1000 ml culture solution, 5.94 g HEPES and 2.0 g sodium bicarbonate were added. It was the basic culture solution. The complete culture solution was added with FBS having a final concentration of 10%, 100U/ml penicillin and 100μg/ml streptomycin. The solution was fully stirred by a magnetic blender, and the pH of the solution was adjusted to 6.8-7.0. The volume was kept constant. After being filtered and disinfected, the solution was

kept into several containers which were sealed and stored at 4℃.

(2) Culture and subculture

The cells were placed in a culture dish followed by the addition of complete culture liquid RPMI1640 and then cultured in a incubator comprising 5% CO_2 at 37℃. In the exponential growth phase, the cells grew vigorously. If the culture liquid became yellow, the liquid should be replaced instantly.

Prior to the plateau phase, the cells needed to be subcultured. During the passage of the anchorage-dependent cells, the old culture liquid was removed. A proper amount of trypsin operating solution was added to digest the cells. When the cells became round and began to fall off, the complete culture liquid was added to terminate the digestion. The cells on the wall were blown off using a pipette. The cells were centrifugalized in a centrifuge tube at 1000 rpm for 5 minutes, and the supernatant was removed. The cells were blown off by the addition of culture liquid and then inoculated in the culture dish at a cell concentration of from 1/10 to 1/20. During the passage of the suspended or semi-suspended cells, the cells were blown homogenous by using a pipette and directly inoculated in a new culture liquid at a concentration of from 1/10 to 1/20.

(3) The frozen and recovery of the cells

The cells in the exponential phase (the anchorage-dependent cells were digested by trypsin at first and then collected by centrifugalization, and the suspended and semi-suspended cells were directly collected by centrifugalization) were added into a pre-formulated cell freezing solution which was stored at 4℃, and were blown to form a unicellular suspension. The concentration of the suspension was adjusted to 10^6 to 5×10^6 cell/ml, and then the

suspension was transferred to a freezing tube surround by cloth. The tube was placed at 4℃, -20℃, and -80℃ respectively and finally suspended under liquid nitrogen such that the temperature of the tube would be lowered gradually. The tube was placed into a liquid nitrogen tank the next day.

During recovery, the freezing tube was taken out of the liquid nitrogen tank and quickly placed into a water bath at 37℃. The tube was stirred continuously such that the cells were quickly defrosted. The cells were transferred to a glass centrifuge tube at a sterile air bench followed by the addition of a small amount of culture liquid to wash the cells. The cells were centrifugalized at 1000 rpm. After the supernatant was removed, the cells were washed once again and then transferred to a culture dish to be cultured in a CO_2 incubator.

(4) Morphologic observations of the growth condition of tumor cell strains

The cells were cultured at a 96-pore culture plate while the cells were in the logarithmic growth phase. While samples having a preset concentration to be tested were placed at the plate, an equivalent amount of dissolvent for dissolving the medicament was added to serve as a comparison. The cells treated by the medicament and the control cells were observed in an optical microscope at different times. The morphologic changes of the cells were recorded and photographed.

(5) Measurement of IC_{50} value by MTT method

The cells, at a concentration of 10^5/ml, were inoculated at a 96-pore culture plate. In each pore, 100 μl cells were inoculated. The cells were placed in a CO_2 incubator until the cells were in the

logarithmic growth phase. The samples to be tested were added according to the preset concentration gradient. For each gradient, the test was conducted at least three times. An equivalent amount of solvent for dissolving the samples was added in the control group. After 48 h of culture, each pore was added 20 μl MTT (5mg/ml). The cells were cultured at 37°C for 4 h. After the supernatant was carefully removed, 100 μl DMSO was added in each pore. The plate was agitated for about 10 minutes such that the precipitates were dissolved. After that, OD value was identified with a Microplate Reader at the wavelength of 490 nm. The survival rate of the cells at various concentrations was calculated according to the following formula:

Survival rate % = average OD value of the sample group/average OD value of the control group $\times$ 100%

Plots of log survival rate of the cells vs. concentration of the medicament were constructed, and the IC_{50} value of each sample was calculated according to graphing methods.

3. Results

3.1 Morphologic observations of the growth conditions of the tumor cell strains

See figure 12 for the morphologic observations of the growth conditions of the tumor cells.

It can be seen from the figure that the cell configuration was significantly changed after human liver cancer HepG2 cells were affected by β-carboline derivatives.

3.2 IC_{50} value measured by MTT methods

IC_{50} value of 1,7,9-trisubstituted β-carboline derivatives

IC_{50} values of 1,7,9-trisubstituted β-carboline derivatives against tumor cell lines are shown in table 35.

Table 35 The IC_{50} of 1,7, 9-trisubstituted β-carboline derivatives against tumor cell lines (μmol/ml)

Cell strains / Compd	PLA-801	HepG2	Bel7402	BGC823	Hela	Lovo	NIH3T3
1	0.05	0.05	0.02	0.07	0.06	0.07	0.06
2	0.03	0.02	0.06	0.05	0.01	0.04	0.04
3	0.02	0.01	0.03	0.05	0.02	0.17	0.07
4	0.11	0.06	0.06	0.07	0.03	0.09	0.04
5	0.08	0.31	0.24	0.20	0.03	0.03	0.08
6	0.06	0.09	0.06	0.10	0.27	0.05	0.05
7	0.42	0.05	0.04	0.02	0.04	0.04	0.05
8	0.05	0.04	0.04	0.05	0.02	0.03	0.02

2. IC_{50} values of 3- and 1,3-disubstiuted β-carboline derivatives

See table 36 for the IC_{50} values of 3- and 1,3-disubstituted β-carboline derivatives against tumor cell lines.

Table 36 The IC_{50} values of 3- and 1,3-disubstituted β-carboline derivatives against tumor cell lines (μmol/ml)

Cell strains	PLA-801	HepG2	Bel7402	BGC823	Hela	Lovo	NIH3T3

Compd							
9*	0.31	2.51	0.30	0.29	0.10	0.17	0.20
10*	0.28	1.25	2.91	1.28	0.06	1.01	0.08
11*	0.26	0.09	0.23	0.11	0.10	0.08	0.10
12*	>3.0	>3.0	>3.0	>3.0	>3.0	1.57	>3.0
13*	0.67	1.06	>3.0	>3.0	>3.0	0.74	0.75
14*	1.90	1.18	>5.0	1.68	0.32	2.48	0.49
15*	>3.0	>3.0	>3.0	>3.0	>3.0	>3.0	>3.0
16*	1.02	>5.0	1.08	0.97	0.15	0.62	2.18
17	0.30	0.28	0.23	0.28	0.22	0.17	0.06
18	0.68	0.27	0.31	0.39	0.29	0.10	0.15
19	0.14	0.08	0.16	0.08	0.09	0.10	0.20
20	0.74	0.36	1.43	0.74	0.40	0.31	0.32
21	0.57	0.34	1.57	0.86	0.31	0.24	0.29
22	0.20	0.23	0.13	0.21	0.17	0.16	0.32
23	2.16	>3.0	>3.0	>3.0	>3.0	>3.0	>3.0
24	1.08	0.58	0.74	0.78	0.62	0.49	0.36
25	0.78	1.13	0.59	0.64	0.78	0.25	0.31

3. IC$_{50}$ values of 3,9-disubstituted β-carboline derivatives

See table 37 for the IC$_{50}$ values of 3,9-disubstituted β-carboline derivatives against tumor cell lines.

Table 37   The IC$_{50}$ values of 3,9-disubstituted β-carboline derivatives against tumor cell lines （μmol/ml）
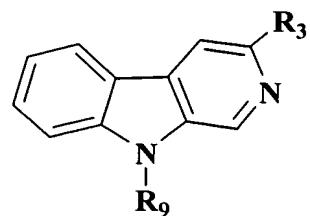
Compd	R_3	R_9	PLA-801	HepG2	Bel7402	BGC823	Hela	Lovo	NIH3T3
26	$COOCH_3$	CH_3	0.12	0.12	0.19	0.18	0.18	0.18	0.09
27	$COOCH_3$	C_2H_5	0.29	0.16	0.53	0.25	0.21	0.11	0.13
28	$COOCH_3$	$n-C_4H_9$	0.14	0.46	0.43	0.31	0.29	0.17	0.08
29	$COOCH_3$	$CH_2 C_6H_5$	0.48	1.77	0.06	0.95	0.12	0.31	0.36
30	$COOC_2H_5$	CH_3	0.12	0.15	0.14	0.15	0.18	0.10	0.10
31	$COOC_2H_5$	C_2H_5	0.17	0.11	0.26	0.14	0.14	0.07	0.10
32	$COOC_2H_5$	$n-C_4H_9$	0.07	0.48	0.78	0.76	0.49	0.17	0.06
33	$COOC_2H_5$	$CH_2 C_6H_5$	0.17	0.08	0.34	0.15	0.01	0.12	0.05
34	$COOH$	CH_3	0.27	0.15	0.33	0.13	0.09	0.09	0.13
35	$COOH$	C_2H_5	0.21	0.18	0.23	0.16	0.18	0.06	0.14
36	$COOH$	$n-C_4H_9$	0.09	0.17	0.09	0.12	0.06	0.01	0.02
37	$COOH$	$CH_2 C_6H_5$	0.10	0.10	0.11	0.05	0.09	0.04	0.05
38	$COOC_4H_9$	CH_3	>3.0	1.61	>3.0	>3.0	>3.0	2.52	>3.0
39	$COOC_4H_9$	C_2H_5	0.11	0.07	0.07	0.08	0.07	0.08	0.08
40	$COOC_4H_9$	$n-C_4H_9$	2.12	1.08	1.08	0.07	0.95	0.04	0.05
41	$COOC_4H_9$	$CH_2 C_6H_5$	0.99	>3.0	>3.0	>3.0	>3.0	1.08	>3.0
42	CH_2OH	$CH_2 C_6H_5$	0.16	0.09	0.11	0.11	0.12	0.10	0.09
43	CH_2OOCCH_3	$CH_2 C_6H_5$	0.25	0.30	0.13	>3.0	0.50	>3.0	>3.0
44	$COOCH_2C_6H_5$	$CH_2 C_6H_5$	>3.0	>3.0	>3.0	>3.0	>3.0	>3.0	>3.0
45	$COOC_2H_5$	$(CH_2)_3 C_6H_5$	>3.0	>3.0	0.387	>3.0	>3.0	>3.0	>3.0
46	$COOC_2H_5$	$CH_2 C_6F_5$	0.05	0.12	0.01	0.05	0.98	0.04	0.06
47	$COOC_2H_5$	$CH_2COC_6H_5$	>3.0	>3.0	1.02	>3.0	>3.0	>3.0	>3.0
48	$COOH$	$(CH_2)_3 C_6H_5$	0.26	0.03	0.05	0.03	0.10	0.01	0.03
49	$COOH$	$CH_2 C_6F_5$	0.09	0.03	0.06	0.04	0.04	0.01	0.02
50	$COOH$	$CH_2COC_6H_5$	0.14	0.09	0.14	0.06	0.07	0.07	0.05
51	$CONHNH_2$	C_2H_5	0.51	0.26	0.37	0.11	0.24	0.19	0.51
52	$CONHNH_2$	$CH_2 C_6H_5$	0.52	0.14	0.06	0.27	0.25	0.25	0.22
53	$NHCOOCH_3$	C_2H_5	>3.0	>3.0	>3.0	>3.0	>3.0	>3.0	>3.0
54	$NHCOOC_2H_5$	C_2H_5	>3.0	>3.0	1.68	>3.0	>3.0	>3.0	>3.0

| 55 | NHCOOC$_2$H$_5$ | CH$_2$ C$_6$H$_5$ | 0.52 | >3.0 | 2.44 | >3.0 | >3.0 | 2.13 | 1.79 |

4. IC$_{50}$ values of 1,3,9-trisubstituted β-carboline derivatives

See table 38 for the IC$_{50}$ values of 1,3,9-trisubstituted β-carboline derivatives against tumor cell lines.

Table 38   The IC$_{50}$ values of 1,3,9-trisubstituted β-carboline derivatives against tumor cell lines (μmol/ml)

Cell strains Compd	PLA-801	HepG2	Bel7402	BGC823	Hela	Lovo	NIH3T3
56	0.14	0.10	0.49	0.13	0.11	0.05	0.06
57	0.16	0.08	0.88	0.07	0.06	0.05	0.06
58	1.39	2.00	0.21	2.08	0.25	0.17	1.82
59	0.11	0.04	0.18	0.04	0.04	0.02	0.03
60	0.05	0.03	0.10	0.004	0.02	0.04	0.04
61	0.16	0.07	0.46	0.09	0.06	0.05	0.09
62	2.35	1.38	1.50	0.64	1.11	0.44	0.63
63	2.33	2.80	1.82	1.66	0.97	0.59	0.71
64	0.41	0.28	0.69	0.28	0.37	0.20	0.14
65	0.21	0.14	0.56	0.27	0.22	0.05	0.11
66	0.19	0.12	0.12	0.16	0.11	0.08	0.08
67	2.00	2.23	>3.0	1.52	2.48	1.34	1.46
68	0.14	0.10	0.46	0.11	1.36	0.06	0.08
69	0.71	0.11	0.33	0.23	0.09	0.10	0.10
70	0.16	0.79	1.01	1.01	0.81	1.17	1.57
71	0.26	0.12	0.91	1.32	0.06	0.06	0.19
72	1.40	1.17	>3.0	0.54	0.96	0.21	0.57
73	0.71	0.54	0.57	0.39	0.42	0.26	0.39

74	0.27	0.17	0.18	0.27	0.16	0.14	0.16
75	0.23	0.13	0.20	0.24	0.14	0.08	0.11
76	>3.0	>3.0	0.62	>3.0	>3.0	>3.0	>3.0
77	>3.0	>3.0	0.38	>3.0	2.66	0.070	0.140
78	0.65	0.16	0.28	0.21	0.17	0.11	0.15
79	0.11	0.16	0.14	0.25	0.14	0.10	0.12

5. IC$_{50}$ values of 9-substituted β-carboline derivatives

See table 39 for the IC$_{50}$ values of 9-substituted β-carboline alkaloid derivatives against tumor cell lines.

Table 39   The IC$_{50}$ values of 9-substituted β-carboline derivatives against tumor cell lines （μmol/ml）

Cell strains / Compd	PLA-801	HepG2	Bel7402	BGC823	Hela	Lovo	NIH3T3
80	0.02	0.23	0.38	0.27	0.35	0.04	0.02
81	0.22	0.10	0.30	0.20	0.33	0.22	0.12
82	0.13	0.17	0.31	0.17	0.23	0.16	0.10
83	0.13	0.10	0.16	0.10	0.09	0.13	0.08
84	0.12	0.11	0.08	0.07	0.05	0.12	0.11

6. IC$_{50}$ values of 2,9-disubstituted β-carboline derivatives

See table 40 for the IC$_{50}$ values of 9-substituted β-carboline derivatives against tumor cell lines.

Table 40 The IC$_{50}$ values of 2,9-disubstituted β-carboline derivatives against tumor cell lines （μmol/ml）

Cell strains / Compd	PLA-801	HepG2	Bel7402	BGC823	Hela	Lovo	NIH3T3
85	0.03	0.02	0.04	0.04	0.03	0.03	0.02
86	0.01	0.04	0.05	0.04	0.03	0.02	0.02
87	0.28	0.20	0.37	0.26	0.07	1.82	0.38
88	1.20	0.43	1.31	1.31	0.86	2.15	0.63
89	0.10	0.03	0.07	0.06	0.02	0.03	0.03

Example 123 Assay of anti-tumor activity

1 Materials and methods

1.1. Materials

(1) Chemicals

Compounds 10, 11, 14, 15, 16, 31, 33, 37, 36, 42, 48, 55, 84 and 86 (see tables 12-3 for the chemical structures), and cyclophosphamide for injection used for positive control sample (available in Shanghai Hualian Pharmaceutical Group) was used.

(2) Animals

Mice $C_{57}BL/6$ and Kunming mice, (provided by Shanghai Experimental Animal Center, the Chinese Academy of Sciences, Certificate No.: Hudonghezhengzidi 107), weighing 18-20 g, The mice for each group of tests could be of the same sex. There was one group of 8 to 10 mice $C_{57}BL/6$ and Kunming mice for the anti-tumor tests and two groups of mice for negative control.

(3) Sources of tumors: Lewis lung cancer cells and S180 sarcoma in mice, which were sub-cultured and maintained by Pharmacological Center of Shanghai Pharmaceutical Industrial Research Institute.

(4) Solvents: physiological saline, and 0.5% CMC-Na solution

1.2 Methods
(1) Setting of the dosage
The medicaments tested were divided into a high dosage group and a low dosage group, which were respectively based on 1/5 and 1/10 of the LD_{50} value or MTD value of said medicaments that were intraperitoneally administered once.

(2) Formulation of the medicament
All samples were weighed and added a small amount of Tween-80 to facilitate dissolution during the experiment, and then 0.5% CMC-Na solution was gradually added to achieve the desired concentration. The volume for experiment was 0.5 ml/ 20 g mouse.

(3) Administration scheme
The medicaments were intraperitoneally administered once a day continuously for 10 days.

For the negative control group, a vehicle of the same volume was intraperitoneally administered once a day continuously for 10 days. For the positive control group, CTX was administered in the dosage of 30 mg/kg once a day continuously for 7 days.

(4) Procedures of assay of the anti-tumor activity in mice
In vivo anti-tumor subcutaneous vaccination in the axilla was employed. Wildly grown tumor source was taken under sterile conditions to form a cell suspension of about 1×10^7 /ml by homogenization method. For a corresponding mouse host, the suspension was injected in the axilla in an amount of 0.2 ml/mouse. The next day, the medicaments were administered according to the

design of the tests. About three weeks later, all the groups of animals were executed. The tumors were taken from the animals and weighed. The inhibition rate of tumors was calculated according to the following formula:

$$(C-T) \ /C \times 100$$

T: average tumor weight of treated group; C: average tumor weight of negative control group.

After the administration of the medicaments, mice were observed immediately for any gross behavioral changes and deaths as well as any neurotoxic symptoms, such as jumping, quivering, twisting and so on.

Table 41 The antitumor activity of β-carboline derivatives against

mouse Lewis lung cancer

Compd	Dosage (mg/ml)	Administration scheme	Number of animals (beginning/end)	Body weight of the animals(g) (beginning/end)		Weight of the tumor X±SD (g)		Inhibition rate (%)	
				First time	Second time	First time	Second time	First time	Second time
10	100	ipx10qd	8/8	20.3/2 3.6	--	1.41±0.21***	--	41.98	--
	50	ipx10qd	8/8	20.1/2 3.5	--	1.63±0.16***	--	32.92	--
11	50	ipx10qd	8/8	18.3/2 3.0	19.8/23.8	1.40±0.16***	1.35±0.14***	44.22	42.8
	25	ipx10qd	8/8	18.7/2 3.4	19.9/24.5	1.78±0.16	1.58±0.09***	29.08	33.05
14	100	ipx10qd	8/8	20.7/2 3.1	--	1.60±0.19***	--	34.16	--
	50	ipx10qd	8/8	20.1/2 3.8	--	1.76±0.25	--	27.57	--
15	100	ipx10qd	8/8	20.3/2 4.0	--	1.65±0.17***	--	32.10	--
	50	ipx10qd	8/8	20.4/2 3.9	--	1.81±0.28	--	25.51	--

17	100	ipx10qd	8/8	19.0/24.8	20.4/24.9	1.84±0.48***	1.51±0.09***	37.20	37.86
	50	ipx10qd	8/8	19.2/25.4	20.2/25.4	2.01±0.28***	1.75±0.09***	31.40	27.98
31	100	ipx10qd	8/8	20.5/23.7	--	1.55±0.22***	--	36.21	--
	50	ipx10qd	8/8	20.6/24.3	--	1.70±0.12***	--	30.04	--
33	100	ipx10qd	8/8	19.9/24.7	19.0/24.5	1.39±0.15***	1.66±0.12***	42.80	43.34
	50	ipx10qd	8/8	20.3/25.4	18.9/24.9	1.60±0.09***	2.02±0.18***	34.16	31.06
36	100	ipx10qd	8/8	19.3/25.2	20.1/25.0	1.70±0.17***	1.29±0.13***	41.98	46.91
	50	ipx10qd	8/8	18.8/25.6	20.1/25.3	2.00±0.28***	1.66±0.17***	31.74	31.69
37	50	ipx10qd	8/8	18.9/23.9	19.5/24.3	1.53±0.14***	1.34±0.14***	39.04	43.22
	25	ipx10qd	8/8	18.6/24.1	19.6/24.9	1.83±0.17	1.67±0.16	27.09	29.24
42	100	ipx10qd	8/8	20.3/25.1	18.9/25.1	1.62±0.12***	1.92±0.26***	33.33	34.47
	50	ipx10qd	8/8	20.0/24.7	19.1/24.7	2.14±0.26	1.89±0.14	22.22	26.96
48	100	ipx10qd	8/8	19.8/24.8	19.3/25.2	1.58±0.08***	1.77±0.15***	34.98	39.59
	50	ipx10qd	8/8	20.1/25.3	19.0/25.0	1.82±0.10	2.09±0.19	25.10	28.67
55	100	ipx10qd	8/8	19.1/23.5	19.7/23.9	1.76±0.10***	1.63±0.18***	29.88	30.93
	50	ipx10qd	8/8	18.7/23.8	19.6/24.5	1.96±0.12***	1.78±0.19***	21.91	24.58
84	100	ipx10qd	8/8	18.5/24.2	19.4/24.7	1.63±0.11***	1.48±0.15***	35.06	37.29
	50	ipx10qd	8/8	19.0/24.1	19.7/25.2	1.83±0.11	1.63±0.16***	27.09	30.93
86	20	ipx10qd	8/8	20.0/25.2	18.7/23.9	1.42±0.11***	1.61±0.17***	41.56	45.05
	10	ipx10qd	8/8	20.2/24.5	19.1/25.1	1.58±0.11***	1.84±0.17***	34.98	37.20
CTX	30	Ivx7qd	8/8	20.1/21.3	18.9/21.3	0.32±0.12***	0.27±0.11***	86.83	90.82
CTX	30	Ivx7qd	8/8	18.8/20.2	19.8/21.0	0.28±0.14***	0.32±0.13***	88.69	86.44
negative	vehicle	Ivx10qd	16/16	20.2/2	19.1/25.5	2.43±0.25	2.93±0.29	--	--

control				5.9					
negative control	vehicle	Ivx10qd	16/16	18.9/24.2	19.7/25.8	2.51±0.24	2.36±0.23	--	--

Note 1: *** represents that P<0.01 compared with the negative control group.

Table 42 The antitumor activity of β-carboline derivatives against mouse

S180 sarcoma

Compd	Dosage (mg/ml)	Administration scheme	Number of animals (beginning/end)	Body weight of the animals(g) (beginning/end)		Weight of tumor X±SD (g)		Inhibition rate(%)	
				First time	Second time	First time	Second time	First time	Second time
10	100	ipx10qd	8/8	20.1/25.5	--	1.56±0.17***	--	40.46	--
	50	ipx10qd	8/8	20.0/26.4	--	1.81±0.20***	--	30.92	--
11	50	ipx10qd	10/10	19.5/25.9	20.6/23.9	1.59±0.16***	1.62±0.38***	44.21	43.16
	25	ipx10qd	10/10	19.5/26.3	20.4/24.2	1.86±0.18***	1.94±0.16***	34.74	31.93
14	100	ipx10qd	8/8	19.9/25.5	--	1.78±0.20***	--	32.06	--
	50	ipx10qd	8/8	19.8/26.7	--	1.98±0.28	--	24.43	--
15	100	ipx10qd	8/8	19.9/26.0	--	1.69±0.16***	--	35.50	--
	50	ipx10qd	8/8	19.8/26.4	--	2.00±0.16	--	23.66	--
17	100	ipx10qd	10/10	19.3/24.2	18.6/25.3	1.73±0.16***	1.81±0.20***	35.21	32.96
	50	ipx10qd	10/10	19.2/24.7	18.5/25.9	2.03±0.24	2.02±0.21	23.97	25.19
31	100	ipx10qd	8/8	20.0/26.1	--	1.65±0.16***	--	37.02	--
	50	ipx10qd	8/8	19.7/26.7	--	1.88±0.21	--	28.04	--
33	100	ipx10qd	10/10	19.4/24.6	18.6/25.1	1.53±0.15***	1.62±0.30***	42.70	40.00

	50	ipx10qd	10/10	19.4/24.4	18.7/25.6	1.78±0.17***	1.83±0.19***	33.33	32.22
36	100	ipx10qd	10/10	19.0/24.5	18.7/25.5	1.52±0.15***	1.62±0.12***	43.07	40.00
	50	ipx10qd	10/10	19.1/24.9	18.3/25.3	1.87±0.16***	1.86±0.28***	29.96	31.11
37	50	ipx10qd	10/10	19.7/26.3	20.6/24.7	1.87±0.13***	1.91±0.23***	34.39	32.98
	25	ipx10qd	10/10	19.6/26.9	20.4/25.1	2.17±0.18	2.16±0.27	23.86	24.21
42	100	ipx10qd	10/10	19.2/24.3	18.9/25.1	1.92±0.25	2.02±0.36	28.09	25.15
	50	ipx10qd	10/10	19.5/24.7	18.4/25.6	2.16±0.38	2.14±0.23	19.10	20.74
48	100	ipx10qd	10/10	19.4/24.8	18.5/26.0	1.84±0.11***	1.83±0.16***	31.09	32.22
	50	ipx10qd	10/10	19.4/24.8	18.6/25.8	2.15±0.39	2.11±0.25	19.48	21.85
55	100	ipx10qd	10/10	19.4/26.7	20.3/24.9	2.02±0.22***	2.05±0.17***	29.12	28.07
	50	ipx10qd	10/10	19.8/27.0	20.9/24.8	2.18±0.23	2.28±0.18	23.51	20.00
84	100	ipx10qd	10/10	19.7/26.4	20.2/25.0	1.79±0.27***	1.79±0.16***	37.19	37.19
	50	ipx10qd	10/10	19.6/26.8	20.4/25.4	2.01±0.24***	1.96±0.16***	29.47	31.23
86	20	ipx10qd	10/10	19.0/23.7	18.9/24.4	1.63±0.26***	1.59±0.17***	38.95	41.11
	10	ipx10qd	10/10	19.3/24.5	18.7/24.9	1.84±0.16***	1.92±0.20	31.09	28.89
CTX	30	ipx7qd	10/10	19.4/20.9	19.8/23.2	0.33±0.14***	0.36±0.13***	87.55	87.37
CTX	30	ipx7qd	10/10	18.7/22.3	20.3/20.9	0.33±0.12***	0.27±0.13***	87.93	90.53
negative control	vehicle	ivx10qd	20/20	19.2/24.6	19.6/27.0	2.67±0.30	2.85±0.33	--	--
negative control	vehicle	ivx10qd	10/10	18.8/25.9	20.5/25.3	2.70±0.31	2.85±0.33	--	--

Note 1: *** represents that P<0.01 compared with the negative control group.

2. Results

2.1 Observations of ordinary symptoms

After the administration of all 14 compounds tested, no symptoms of nervous toxicity, such as quivering, jumping and twisting, occurred. It was thus demonstrated that all compounds do not have nervous toxicity.

2.2 Results of the anti-tumor activity

The results of the therapeutic effect on Lewis lung cancer cells are shown in table 41. See figure 13 for the real image of the therapeutic effect. The results of the therapeutic effect on S180 sarcoma are shown in table 42. See figure 14 for the real image of the therapeutic effect.

For the sake of comparison, the test results of the *in vivo* anti-tumor therapeutic effect are shown in table 43.

It can be seen from the results shown in table 43 that (1) among all 22 compounds tested, there are 7 compounds (compounds 4, 6, 10, 11, 33, 36 and 86) that exhibit an inhibition rate of tumors greater than 40% against both Lewis lung cancer cells and S180 sarcomata, and there are two compounds (compounds 3 and 37) that exhibit an inhibition rate of tumors greater than 40% merely against Lewis lung cancer cells; (2) compounds 6 and 36 have the strongest anti-tumor effect on Lewis lung cancer cells and have an inhibition rate of up to 46.9%; (3) among all 22 compounds tested, except compounds 14, 15, 27 and 55 have an anti-tumor activity against Lewis lung cancer cells a little worse than or equivalent to that of harmines (compound 1), all the other compounds anti-tumor

activity is higher than that of harmines, and (4) Lewis lung cancer cells are more sensitive to the anti-tumor activity of β-carboline alkaloid derivatives than S180 sarcoma.

Table 43　　The antitumor activity of β-carboline derivatives in vivo
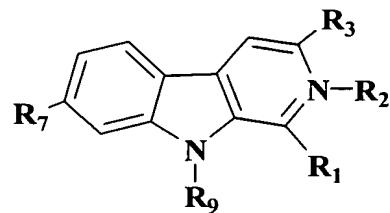
| Compd | R_1 | R_2 | R_3 | R_7 | R_9 | Inhibition rate (%) | |
						Lewis cancer	S180 sarcomata
1*	CH_3	H	H	CH_3O	H	34.1	15.3
2*	CH_3	H	H	CH_3O	CH_3	38.1	32.1
3*	CH_3	H	H	CH_3O	C_2H_5	42.0	37.6
4*	CH_3	H	H	CH_3O	$n-C_4H_9$	44.0	40.9
6*	CH_3	H	H	CH_3O	$CH_2C_6H_5$	46.9	45.2
10	H	H	$CO_2C_2H_5$	H	H	41.98	40.46
11	CH_3	H	$CO_2C_2H_5$	H	H	44.22	44.21
14	C_6H_5	H	CO_2CH_3	H	H	32.06	34.16
15	$C_6H_5-p-OCH_3$	H	CO_2CH_3	H	H	35.50	32.10
16	C_6H_5-p-OH	H	CO_2CH_3	H	H	37.86	32.96
17*	H	H	COOH	H	H	33.4	32.2
26*	H	H	CO_2CH_3	H	CH_3	35.0	31.1
27*	H	H	CO_2CH_3	H	C_2H_5	30.5	29.0
31	H	H	$CO_2C_2H_5$	H	C_2H_5	37.02	36.21
33	H	H	$CO_2C_2H_5$	H	$CH_2C_6H_5$	43.3	42.11
36	H	H	COOH	H	$n-C_4H_9$	46.91	43.07
37	H	H	COOH	H	$CH_2C_6H_5$	43.22	34.39
42	H	H	CH_2OH	H	$CH_2C_6H_5$	34.47	28.09
48	H	H	COOH	H	$(CH_2)_3C_6H_5$	39.59	32.22
55	H	H	$NHCO_2C_2H_5$	H	$CH_2C_6H_5$	30.93	29.12
84	H	H	H	H	$CH_2C_6H_5$	37.29	37.19
86	H	$CH_2C_6H_5$	$CO_2C_2H_5$	H	$CH_2C_6H_5$	41.56	41.11

Example 124 DNA photocleavage effect of β-carboline derivatives

Materials and methods
1. Materials
(1) Instruments
DYY-2C electrophoresis apparatus (Beijing Liuyi Instrument Factory), DYCP-31D electrophoresis cell (Beijing Liuyi Instrument Factory) and gel image system was used.

(2) Reagents
Plasmid pGBK (8.0 Kb, stored by this lab), agarose, Tris-HCl buffer solution (pH: 7.5), and E.Z.N.A. plasmid minipreps kit I (OmegaBio-Tek, U.S.) was used.

Chemicals: compounds 1, 2, 3, 4, 5, 6, 7, 8, 11, 13, 17, 34, 35, 36, 37, 56, 57, 58, 68, 69, 70, 80, 81, 82, 83 and 84. See table 1 for the structures of said compounds.

2. Methods
(1) Plasmids pGBK
Plasmid pGBK was prepared using *Escherichia coil* culture and then was purified using an E.Z.N.A. Plasmid Minipreps Kit I. The plasmid was suspended in Tris-EDTA buffer and detected with agarose gel electrophoresis, and then stored at -20℃.

(2) DNA photocleavage examination
The experiments were carried out in a volume of 20μl containing 0.3μg of plasmid pGBK DNA in Tris-HCl buffer (50mM Tris-HCl, pH 7.5) and various harmine derivatives with different concentration. Reaction volumes were held in polyethylene

microcentrifuge tubes, and then irradiated under a mercury-vapor ultraviolet light (8w, 365nm, 5cm distance). Samples were irradiated for 2 h at room temperature. Identical treatments were placed in dark at room temperature. After irradiation, a 2μl of a mixture containing 50% sucrose and 0.25% bromophenol blue was added to the irradiated solution. Samples were analyzed by electrophoresis on 0.7% agarose horizontal slab gel containing 0.5ug/mL-1 ethidium bromide in Tris-EDTA buffer (40mM Tris, 20mM acetic acid, 1mM EDTA, pH 8.0). Untreated pGBK DNA was used as control. Electrophoretic analyses were carried out at 100Vcm-1 for 1 h. Gels were photographed under UV light with Bio-Rad digital camara.

3. Results

According to the ratio of the circular nicked DNA formed under such conditions to the supercoiled DNA, the photo-induced DNA cleaving abilities of various β-carboline derivatives were confirmed.

The results are shown in figure 1.

We can primarily arrive at the following structure-activity relationships according to the above results: (1) the photocleavage activity of β-carboline derivatives is dependent on the presence, position and properties of the substituents on the β-carboline ring, and (2) electron-releasing substituents on the β-carboline ring facilitate the photo-induced DNA cleavage ability, whereas electron-withdrawing substituents were detrimental to their DNA cleavage activity.

Example 125 DNA thermal denaturation studies of β-carboline

derivatives

1. Materials

Instruments:UV 2501PC spectrograph (Shimadzu, Japan), SP-752 UV spectrophotometer (installed with constant temperature water bath accessory and analysis software, Shanghai Spectrograph Plant), CT-DNA (Sigma), PE buffer solution (1mM Na_2HPO_4, 0.1mM EDTA, pH 7.6) were used.

Chemicals: 19 β-carboline derivatives: 1, 2, 3, 4, 5, 6, 7, 8, 33, 36, 39, 42, 49, 66, 80, 81, 82, 83, and 84. See table 2 for their structures. Adriamycin hydrochloride and camptothecin (Sigma Company).

2.Methods
(1) Determination of $_\triangle T_m$

Experiments were carried out in PE buffer (1mM Na2HPO4, 0.1mM EDTA, pH 7.6) in a thermostatically controlled cell hold, and the quartz cuvette (1cm path length) was heated by circulating water at a heating rate of 0.5°C/min from 25 to 95°C. Amsacrine and Doxorubicin Hydrochloride were used as standards. In all cases, the concentration of CT-DNA was 15ug/ml. The 'melting' temperature T_m was taken as the mid-point of the hyperchromic transition.

According to the above operational conditions, 20 uM various β-carboline derivatives were added each time, and the changes of the Tm curves prior to the addition of the compounds and after the addition of the compounds were observed. ΔTm value of each compound was calculated according to the following formula:

$$\triangle T_m = T_m{}^{\text{drug-DNA complex}} - T_m{}^{\text{DNA alone}}$$

The $_A$Tm of each compound was calculated, Experiments were repeated three times, and an average value was calculated.

(2) UV absorption spectrum method

CT-DNA (40 ug/ml) was added into a PE suffer solution (pH7.6), and its scan curve was identified by a UV 2501PC UV spectrograph at a wavelength of from 200 to 400 nm. Compounds to be tested (20 uM) were added into the PE buffer solution, and its scan curve was identified by the spectrograph at a wavelength of from 200 to 400 nm. CT-DNA (40 ug/ml) and compounds to be tested (20 uM) were added into the PE buffer solution. The mixture was cultured at 37°C for 2 h, and its scan curve was identified by the UV spectrograph at a wavelength of from 200 to 400 nm. A curve showing that DNA did not react with the medicaments tested was calculated by the addition of the scan curve of CT-DNA with the scan curve of the compounds tested to calculate the OD value at 260 nm of the curve. After that, the OD value at 260 nm of the scan curve of the medicaments tested and that of the CT-DNA mixtureOD value at 260 nm of the scan curve were calculated and compared to determine the influences of the medicaments tested on the UV absorption spectrum of the DNA molecules.


Results

(1) Influences of β-carboline derivatives on the T_m of the CT-DNA

See table 44 for the influences of β-carboline alkaloid derivatives on the Tm value of the CT-DNA, and figure 2 for the graphic exhibition. Under the conditions of these tests, the Tm value of CT-DNA is 61°C. We can see from the figures that β-carboline

derivatives can increase the Tm value of CT-DNA and that
compound 6 has the greatest influence on the Tm value of the
CT-DNA. Compound 6 increased the Tm value of the CT-DNA by
10.3℃, which is equivalent to the insertion effect of adriamycin
($_\Delta$Tm=10.8℃) on the positive control group. The influence of
compound 84 on the Tm value of the CT-DNA is relatively small,
the $_\Delta$Tm value is only 1.5℃. In particular, we also observed in the
tests that the camptothecin for the positive control group and
β-carboline compounds 33, 36 and 42 reduced the Tm of the
CT-DNA. The ΔTm values are respectively −2.5℃ (camptothecin),
-1.2℃ (compound 33), -1.5℃ (compound 36) and −0.3℃
(compound 42).

Table 44 **Effect of binding by β-carboline derivatives on the thermal stability of**

the CT-DNA
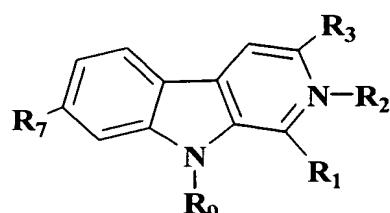
Compounds	R_1	R_2	R_3	R_7	R_9	Δ Tm
1	CH_3	H	H	CH_3	H	8.5
2	CH_3	H	H	CH_3	CH_3	5.3
3	CH_3	H	H	CH_3	C_2H_5	7.3
4	CH_3	H	H	CH_3	n-C_4H_9	6.7
5	CH_3	H	H	CH_3	C_2H_4OH	5.9
6	CH_3	H	H	CH_3	$CH_2C_6H_5$	10.3
7	CH_3	H	H	CH_3	$CH_2C_6F_5$	7.5
8	CH_3	H	H	CH_3	$(CH_2)_3C_6H_5$	7.9
33	CH_3	H	$COOC_2H_5$	H	$CH_2C_6H_5$	-1.2

36	H	H	COOH	H	n-C_4H_9	-1.5
39	H	H	$COOC_4H_9$	H	C_2H_5	1.6
42	H	H	CH_2OH	H	$CH_2C_6H_5$	-0.3
80	H	H	H	H	H	3.1
81	H	H	H	H	CH_3	3.3
82	H	H	H	H	C_2H_5	4.2
83	H	H	H	H	n-C_4H_9	2.4
84	H	H	H	H	$CH_2C_6H_5$	1.5
Adriamycin						10.8
Camptothecin						-2.5

(2) Effect of absorbance by β-carboline derivatives on the UV spectrum of the CT-DNA.

See figure 3 for the influence of β-carboline derivatives on the UV absorption spectrum of CT-DNA. It can be seen from the figure that (1) compounds 1, 3, 4, 6, 82 and 83 and the adriamycin can reduce the UV absorption of DNA, and the influence of adriamycin is the most significant, followed by compound 3; and (2) on the contrary, compounds 36, 37, 43, 49 and 66 and the camptothecin can increase the UV absorption of DNA, and the influence of compound 36 on the increase of UV absorption value of the DNA is the most obvious.

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